Preliminary Report: Analysis of the baseline study on the prevalence of Salmonella in laying hen flocks of Gallus gallus

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Preliminary Report

Analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of *Gallus gallus*

This report is based on the dataset of the baseline study updated on 7 April 2006.

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Key

List of country abbreviations

EU Member State	EU Member State abbreviation	EU Member State code
Austria	AT	1
Belgium	BE	2
Cyprus	CY	3
Czech Republic	CZ	4
Denmark	DK	5
Estonia	EE	6
Finland	FI	7
France	FR	8
Germany	DE	9
Greece	EL	10
Hungary	HU	11
Ireland	IE	12
Italy	IT	13
Latvia	LV	14
Lithuania	LT	15
Luxembourg	LU	16
Malta	MT	17
Poland	PL	18
Portugal	PT	19
Slovakia	SK	20
Slovenia	SI	21
Spain	ES	22
Sweden	SWE	23
The Netherlands	NL	24
United Kingdom	UK	25
EU	the 25 EU Member States, taken together	
EEA member	EEA country abbreviation	
Norway	NO	26

List of abbreviations

CI	Confidence interval
EEA	European Economic Area
EU	European Union
MS	Member State
SE	*Salmonella* Enteritidis
STM	*Salmonella* Typhimurium

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Acknowledgements

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1. Summary

An EU-wide *Salmonella* baseline study was conducted on commercial large-scale laying hen holdings with at least 1,000 laying hens in the flock. The study was carried out in all the Member States, and the sampling of the holdings took place during the period of 1 October 2004 to 30 September 2005. Norway participated in the study on a voluntary basis.

The aim of the study was to estimate the *Salmonella* holding observed prevalence at the global EU-level as well as for each Member State specifically. In total, 5,317 laying hen holdings in the EU were included in this study. But a clean dataset comprising 4,797 holdings was mainly used to analyse the results. Samples were taken from flocks of laying hens during the last nine weeks of their production. One flock per each holding was sampled by taking five faeces samples and two dust samples.

The results show that at the global EU-level 20.3% of the large-scale laying hen holdings are bacteriologically positive for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium. The Member States' -specific *Salmonella* Enteritidis – *Salmonella* Typhimurium holding observed prevalence estimates varied largely, from a minimum of 0% to a maximum of 62.5%.

The holding observed prevalence for any *Salmonella* subspecies was, in general, higher. At the global EU-level the presence of any *Salmonella* spp. was detected in 30.7% of the large-scale laying hen holdings. The range of the Member States' -specific *Salmonella* spp. holding observed prevalence was also wide, from a minimum of 0% to a maximum of 79.5%.

The number of positive samples in a holding varied between 1 and 7, and an important proportion of the holdings was found positive on the basis of only one or two positive samples.

Based on preliminary univariate analysis, holdings having *Salmonella* Enteritidis vaccinated flocks were less likely to be positive for *Salmonella* Enteritidis, in eight countries where both flocks vaccinated and unvaccinated against *Salmonella* Enteritidis were sampled. But with the subgroup of *Salmonella* Enteritidis positive holdings in these countries, there was no difference in the proportion *Salmonella* Enteritidis positive samples between vaccinated and unvaccinated flocks. Covered by the clean dataset, dust samples were found more positive for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium than faeces samples. Medication with antibiotics within two weeks prior the sampling did not seem to have an impact on the results for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium.

The five most frequently isolated *Salmonella* serovars in the EU were, in descending order: *Salmonella* Enteritidis, *Salmonella* Infantis, *Salmonella* Typhimurium, *Salmonella* Mbandaka and *Salmonella* Livingstone.

2. Introduction

2.1. Background

This was the first baseline study regarding the prevalence of a zoonotic agent in an animal population ever organised at the EU level.

2.1.1. Legal framework

This baseline study was carried out in accordance with the new Zoonoses legislation, aiming at reducing the incidence of food-borne diseases in the European Union. Regulation EC/2160/2003[1] requires an EU target for reducing *Salmonella* prevalence in laying hens to be laid down, and therefore comparable data on current prevalence in Member States (MSs) needed to be available. To this end, a special baseline study was carried out in order to estimate the prevalence of *Salmonella* spp. in laying hens. The objectives, the sampling frame, the diagnostic testing methods, as well as the collection of data, evaluation and reporting and timelines of this baseline study, are specified in Commission Decision 2004/665/EC[2].

2.2. Objectives of the study

The primary objective of the baseline study was to estimate the prevalence of *Salmonella* spp. in commercial large-scale holdings of laying hens across the European Union.

The global EU holding prevalence as well as the MSs-specific holding prevalence was to be investigated. The variables that needed to be analysed were the presence or absence of any zoonotic *Salmonella* (*Salmonella* spp.) in a flock. In particular, the presence or absence of *Salmonella* Enteritidis or *Salmonella* Typhimurium was to be investigated, as well as the simultaneous presence or absence of these two serovars. For the target for *Salmonella* reduction will cover, in the beginning, at least these two serovars.

The second objective was to investigate the relative sensitivity of both sample types - faeces and environmental samples as well as role of vaccination.

Lastly, additional epidemiological information in relation to *Salmonella* serotypes, flock types, holding/flock sizes and vaccination was also to be obtained.

3. Materials and methods

The essential survey elements are mentioned briefly, because the technical specifications of this study are described in detail in the document "European Commission DG SANCO. Baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus* in the EU: Technical specifications. SANCO/34/2004 Rev3. Working document, 13 July 2004. Presented at the meeting of the Standing Committee on the Food Chain and Animal Health on 15 July 2004[3].

3.1. Survey design

The baseline study was conducted on large-scale laying hens holdings with at least 1,000 laying hens in a flock. It ran from 1 October 2004 to 30 September 2005. Together five pooled samples of faeces and two pooled sampled of dust were collected from each flock with laying hens during the last nine weeks of their production period.

During the study period, at least 172 poultry holdings in each MS (where MSs have more than 172 holdings) were to be sampled in accordance with the technical specifications[4].

The study had a multistage design. At the first stage, holdings were independently selected within all 26 countries. The countries were considered to be strata (i.e. clusters that are sampled separately). At the second stage, one flock was selected within each holding selected. At the third stage, seven samples (five faeces and two dust samples) were taken from the selected flock.

The following observed prevalence parameters were analysed separately:

- *Salmonella* spp.,
- SE or STM or both (SE/STM),
- SE,
- STM.

A holding was considered positive if the presence was detected in at least one of the seven samples taken respectively

- of *Salmonella* spp.,
- of SE or STM or both (SE/STM),
- of SE,
- of STM.

These prevalences were first investigated at the holding-level. A secondary objective was to investigate the sample-level and the within-flock proportion of positive samples.

3.2. Sample size

The number of holdings to be sampled in each country has been calculated considering the total number of holdings with more than 1,000 laying hens, an expected prevalence of 20% and an accuracy of 3%, with a 95% level of confidence. The samples have been distributed proportionally to the number of holdings in each holding size class. Secondly, the within-flock sample-size was set at seven samples.

In order to obtain a number of sampled holdings sufficient to detect a change in prevalence in time, all countries were to sample at least 172 holdings, allowing to detect a decrease in prevalence of at least 10% if the expected prevalence is 20%, alpha=0,05 and test potency=0,95.

For this reason, in those countries in which the number of holdings to be sampled was less than 172, it was necessary to increase the number of holdings to be sampled in order to reach 172 holdings. In these cases, the following scheme was used. In countries having in total more than 172 holdings, additional small flocks (<1000) had to be sampled in order to reach the number of 172 (Ireland, Portugal). In countries which have a total number of holdings less than 172, all the relevant existing holdings were to be sampled: Cyprus (33), Czech Republic (around 90), Estonia (between 20 and 30), Latvia (around 20), Lithuania (around 20), Luxembourg (between 10 and 20), Malta (around 20), Slovak Republic (around 40), Slovenia (104).

3.3. Collection of samples and holding and management characteristics

The samples were taken by official veterinarians or agents of the competent authority and sent to the National Reference Laboratories for *Salmonella* where detection and serotyping took place. In the case that the National Reference Laboratory did not have the capacity to perform all the analyses, or if it was not the laboratory that performed detection routinely, the competent authorities could decide to designate a limited number of other laboratories involved in official control of *Salmonella* to perform the analyses.

The samplers also noted a number sample and holding and management characteristics as specified in the data dictionary.

3.4. Bacteriological testing

The detection method recommended by the Community Reference Laboratory for *Salmonella* in Bilthoven, Netherlands, was used: the method is a modification of ISO 6579 (2002), where a semi-solid medium (MSRV) is used as the single selective enrichment medium. The semi-solid medium is incubated at 41.5 +/- 1 °C for 2x (24+/-3) hours.
At the laboratory, samples were kept refrigerated until examination, which should have been carried out within 48 hours after receipt. The rules for preparation of the samples were described in the technical specifications document[4].

3.5. Methods used to estimate the *Salmonella* observed prevalence

3.5.1. Holding observed prevalence

In this *Salmonella* baseline study, the population of laying hens is finite. Moreover, the sample fraction, i.e. the fraction of sampled laying hen holdings from the total population of laying hen holdings was always above 5%. Consequently, all statistical models and methods were based on the hyper-geometrical law, not the binomial one.

MS-specific holding observed prevalence was estimated by a design-based analysis. When estimating EU holding observed prevalence, weighting was implemented to take into account the size of holding populations in each country.

The 95% confidence intervals for the holding observed prevalence was estimated by linear interpolation on the basis of the normalised cumulative probability of 0.975.

Analyses were performed using the statistical software:
- SAS® 9.1.3 (2002-2003 by SAS Institute Inc., Cary, NC, USA)
- Stata®/SE 9.1 (StataCorp LP, Texas, USA, 2006).
- @Risk® 4.5.5 (Palisade Corporation, 2004).

3.6. Data analysis

3.6.1. Data cleaning

The final dataset that EFSA received from the Commission was checked for non-valid data. Firstly the presence of erroneous values was investigated. These are real data encoding errors, for example a negative age or a sampling date later than the date of bacteriological testing. Secondly the dataset was checked for non-plausible data violating the technical specifications of the study protocol.

The filtering or cleaning of such contents-level non-valid data is necessary to address the primary study objective. To this end a list of data exclusion criteria (see Annex I) was defined identifying such non-valid data in key variables. This query grid was applied in two steps, and the holdings having non-valid or non-plausible data were excluded, which resulted in a clean dataset.

4. Results

4.1. Overview of EFSA's clean and full dataset

On the 2nd of December 2005 EFSA received a first dataset from the Commission, containing 5,125 holdings and 35,875 samples. Upon validation of the contents, it appeared that the dataset comprising all MSs' datasets still had to be upgraded significantly. Moreover, the data sent by MSs needed to be thoroughly validated, as one third of the records had erroneous or non-plausible values.

EFSA's Working Group on the analysis of the *Salmonella* baseline study decided, together with the Commission, that it was opportune for the Commission to ship subsets of data containing erroneous or non-plausible values back to the countries for verification and possible correction. EFSA provided the Commission with these subsets on the 30th of January 2006.

On the 24th of February, EFSA received the final revised dataset from the Commission.

4.1.1. Full dataset

An overview of the sampled holdings contained in the full dataset that EFSA received is given in Annex II.

The final full dataset, structurally validated by the Commission, contained 5,317 EU laying hen holdings. There were no data in this dataset for Malta. Furthermore, 314 holdings from Norway were included.

4.1.2. Clean dataset

An overview of the clean dataset is given in Annex III for the holding-level and in Annex IV for the sample-level. In total it contained 4,561 EU laying hen holdings with 31,927 samples that met the inclusion criteria. There were no data in this dataset for Slovakia since for this country all samples had at least one exclusion value. Moreover also Malta was not included since this country was not included in the full dataset. Furthermore, 236 holdings from Norway were included.

An overview of the number of excluded holdings per MS is given in Table 1. The reasons why samples or holdings were excluded are summarized for every country in Table 2. The criterion that caused the highest number of records to be excluded was criterion number five, which excluded all records from holdings with one reported flock per holding where the reported number of hens in the holding did not equal the reported number of hens in the flock, account taken of an allowed 10% difference.

Table 1. **Overview of the data cleaning, *Salmonella* in laying hens holding in the EU and Norway, 2004 – 2005**

Member States	Actually sampled holdings, structurally validated by COM and sent to EFSA	Actually sampled holdings, validated on contents by EFSA	Number of holdings, which were excluded by EFSA
	N full dataset	N clean dataset	N
AT	349	334	15
BE	150	130	20
CY	25	2	23
CZ	70	64	6
DE	564	522	42
DK	161	85	76
EE	11	11	0
EL	163	107	56
ES	507	481	26
FI	268	249	19
FR	524	511	13
HU	277	267	10
IE	156	146	10
IT	381	295	86
LT	11	8	3
LU	9	9	0
LV	6	6	0
NL	471	392	79
PL	362	290	72
PT	86	44	42
SWE	171	97	74
SI	108	98	10
SK	33	0	33
UK	454	413	41
EU	5,317	4,561	756
NO	314	236	78

Table 2. **Overview of number of samples with non-plausible characteristics in the full dataset *Salmonella* in laying hens holding in the EU and Norway, 2004 – 2005**

	Number of the exclusion criterion																																		
Member States	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17 a**	**17 b**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
	number of samples with non plausible characateristics																																		
AT	0	0	0	0	0	0	0	0	7	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	84
BE	0	0	0	0	49	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	59
CY	0	0	0	7	147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	5	0	0	0	0	0	13
CZ	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	9
DE	0	0	0	7	0	0	0	0	0	266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	14
DK	0	7	0	7	35	14	0	0	14	0	0	0	0	7	0	7	0	0	0	0	0	0	0	0	0	0	1	0	25	0	0	0	0	469	23
EE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EL	0	14	0	7	112	0	0	0	0	14	0	0	0	0	0	7	0	14	0	0	0	0	0	0	0	0	0	0	14	0	89	0	89	0	44
ES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	182
FI	0	0	0	0	0	0	0	0	0	63	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	8
FR	14	0	0	0	0	0	0	14	7	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	77
HU	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	21	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
IE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	23
IT	28	14	0	0	35	0	7	28	7	7	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	1	45	85	0	0	0	0	68
LT	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
LU	0	0	0	0	0	0	7	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0
LV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NL	0	126	0	0	455	0	0	0	0	0	0	0	0	7	0	21	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	49
PL	0	21	7	14	49	0	7	7	14	56	14	0	0	28	0	49	0	189	14	0	0	0	0	0	0	0	1	0	38	0	3	0	3	0	79
PT	0	35	0	0	217	0	0	42	0	21	7	0	0	21	7	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
SWE	0	0	0	7	42	0	0	7	0	7	0	0	0	7	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	470
SI	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	63	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SK	0	0	0	0	0	0	7	0	0	0	0	0	231	0	0	7	42	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	21	4
UK	7	14	7	7	168	0	0	14	0	14	14	0	7	0	0	63	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	14	14
EU-25	49	231	14	70	1,316	14	28	112	49	476	42	0	238	70	7	273	42	231	35	0	0	0	0	0	0	0	11	18	167	85	93	0	93	0	511	1,244
NO	0	7	7	0	28	0	0	0	7	70	0	0	0	7	0	77	0	0	0	0	0	0	0	0	0	0	0	0	1	413	0	0	0	0	0	0

4.2. Features of the European laying hens population

A short overview of the features of the European laying hens population is given in Annex V.

4.3. *Salmonella* observed prevalence

4.3.1. *Salmonella* holding observed prevalence

The *Salmonella* holding observed prevalence for every MS and at EU-level as well as for Norway, is presented in Table 3. A graphical display of those figures is in Figure 1. Analogous figures covered by the full dataset are in Table 4.

The following observations can be made specifically for each outcome variable, based on the clean dataset

***Salmonella* spp. holding observed prevalence**

There were 1,378 holdings in the EU where the presence of *Salmonella* spp. was detected in at least one of the seven samples taken, which resulted in a *Salmonella* spp. EU weighted holding observed prevalence of 30.7% (95% CI=29.6-31.8). The range of *Salmonella* spp. holding observed prevalence in the EU is from a minimum of 0% (LU, SWE) to a maximum of 79.5% (PT).

A graphical display of the 95% CIs of *Salmonella* spp. holding observed prevalence for every MS and at EU-level as well as for NO, ascending ranked based on the point estimates, is in Figure 2.

Based on the full dataset, the *Salmonella* spp. EU weighted holding observed prevalence is 31.7% (95% CI=30.1-32.0). The range of *Salmonella* holding observed prevalence in the EU is from a minimum of 0% (LU, SWE, SK) to a maximum of 77.6% (PL).

The following MS-specific differences between the clean and full dataset prevalence figures are noteworthy: CY, clean 50% - full 28%; EL, clean 37.4% - full 54.6%; PT, clean 79.5% - full 70.9%; SK, clean no data - full 0%.

***Salmonella* Enteritidis / *Salmonella* Typhimurium holding observed prevalence**

There were 927 holdings in the EU where the presence of SE or STM or both SE and STM was detected in at least one of the seven samples, which resulted in a SE-STM EU weighted holding observed prevalence of 20.3% (95% CI=19.4-21.3). The range of the SE-STM holding observed prevalence in the EU is from a minimum of 0% (CY, IE, LU, LV, and SWE) to a maximum of 62.5% (CZ).

A graphical display of the 95% CIs of SE-STM holding observed prevalence for every MS and at EU-level as well as for NO, ascending ranked based on the point estimates, is in Figure 3.

Based on the full dataset, the SE-STM EU weighted holding observed prevalence is 20.4% (95% CI=19.5-21.2). The range of the SE-STM holding observed prevalence in the EU is from a minimum of 0% (IE, LU, LV, SK and SWE) to a maximum of 64.3% (CZ).

The following MS-specific differences between the clean and full dataset prevalence figures are noteworthy: CY, clean 0% - full 8%; EL, clean 22.4% - full 30.1%; LT, clean 50% - full 36.4%; PT, clean 47.7% - full 40.7%; SK, clean no data - full 0%.

Salmonella Enteritidis and *Salmonella* Typhimurium holding observed prevalence

The SE holding observed prevalence and the STM holding observed prevalence, for every MS and at EU-level as well for NO, are presented separately in Annex VI (clean dataset) and in Annex IX (full dataset), respectively.

A graphical display of the 95% CIs of the SE holding observed prevalence and of the STM holding observed prevalence for every MS and at EU-level as well for NO, ascending ranked based on the point estimates, is in Annex VII and in Annex VIII, respectively.

Table 3. ***Salmonella*** **holding observed prevalence in the EU and Norway, 2004 – 2005 (*clean dataset*)**

Member States	number of holdings [1]	*S*. spp.				SE/STM			
	N	N	%	95 CI lower limit	95 CI upper limit	N	%	95 CI lower limit	95 CI upper limit
AT	334	52	15.6	*12.8*	*18.7*	36	10.8	*8.5*	*13.5*
BE	130	46	35.4	*28.9*	*42.2*	34	26.2	*20.4*	*32.7*
CY	2	1	50.0	*7.7*	*88.7*	0	0.0	*0.0*	*67.1*
CZ	64	42	65.6	*61.3*	*68.2*	40	62.5	*58.0*	*65.2*
DE	522	150	28.7	*25.4*	*32.3*	127	24.3	*21.2*	*27.7*
DK	85	2	2.4	*0.7*	*6.8*	1	1.2	*0.1*	*5.1*
EE	11	2	18.2	*18.2*	*18.2*	1	9.1	*9.1*	*9.1*
EL	107	40	37.4	*30.0*	*45.1*	24	22.4	*16.5*	*29.6*
ES	481	352	73.2	*70.0*	*76.0*	248	51.6	*48.2*	*54.8*
FI	249	1	0.4	*0.0*	*1.6*	1	0.4	*0.0*	*1.6*
FR	511	88	17.2	*14.6*	*20.2*	41	8.0	*6.2*	*10.3*
HU	267	117	43.8	*39.9*	*47.6*	90	33.7	*30.0*	*37.4*
IE	146	2	1.4	*0.6*	*2.6*	0	0.0	*0.0*	*0.7*
IT	295	89	30.2	*25.8*	*34.8*	24	8.1	*5.8*	*11.3*
LT	8	4	50.0	*24.7*	*69.4*	4	50.0	*24.7*	*69.4*
LU	9	0	0.0	*0.0*	*0.0*	0	0.0	*0.0*	*0.0*
LV	6	1	16.7	*1.0*	*46.8*	0	0.0	*0.0*	*29.1*
NL	392	62	15.8	*12.9*	*19.2*	31	7.9	*5.9*	*10.5*
PL	290	224	77.2	*72.7*	*81.1*	162	55.9	*50.8*	*60.7*
PT	44	35	79.5	*66.7*	*87.7*	21	47.7	*34.9*	*60.4*
SWE	97	0	0.0	*0.0*	*2.8*	0	0.0	*0.0*	*2.8*
SI	98	19	19.4	*15.4*	*23.8*	9	9.2	*6.4*	*12.7*
UK	413	49	11.9	*9.6*	*14.6*	33	8.0	*6.1*	*10.4*
EU[2]	4,561	1,378	30.2			927	20.3		
EU weighted prevalence			**30.7**	***29.6***	***31.8***		**20.3**	***19.4***	***21.3***
NO	236	0	0.0	*0.00*	*1.2*	0	0.0	*0.00*	*1.2*

[1]: validated on the contents-level by EFSA

[2]: These EU figures do not include data for MT and SK

Figure 1. **Graphical display of the *Salmonella* holding observed prevalence in the EU, 2004 – 2005 (*clean dataset*)**

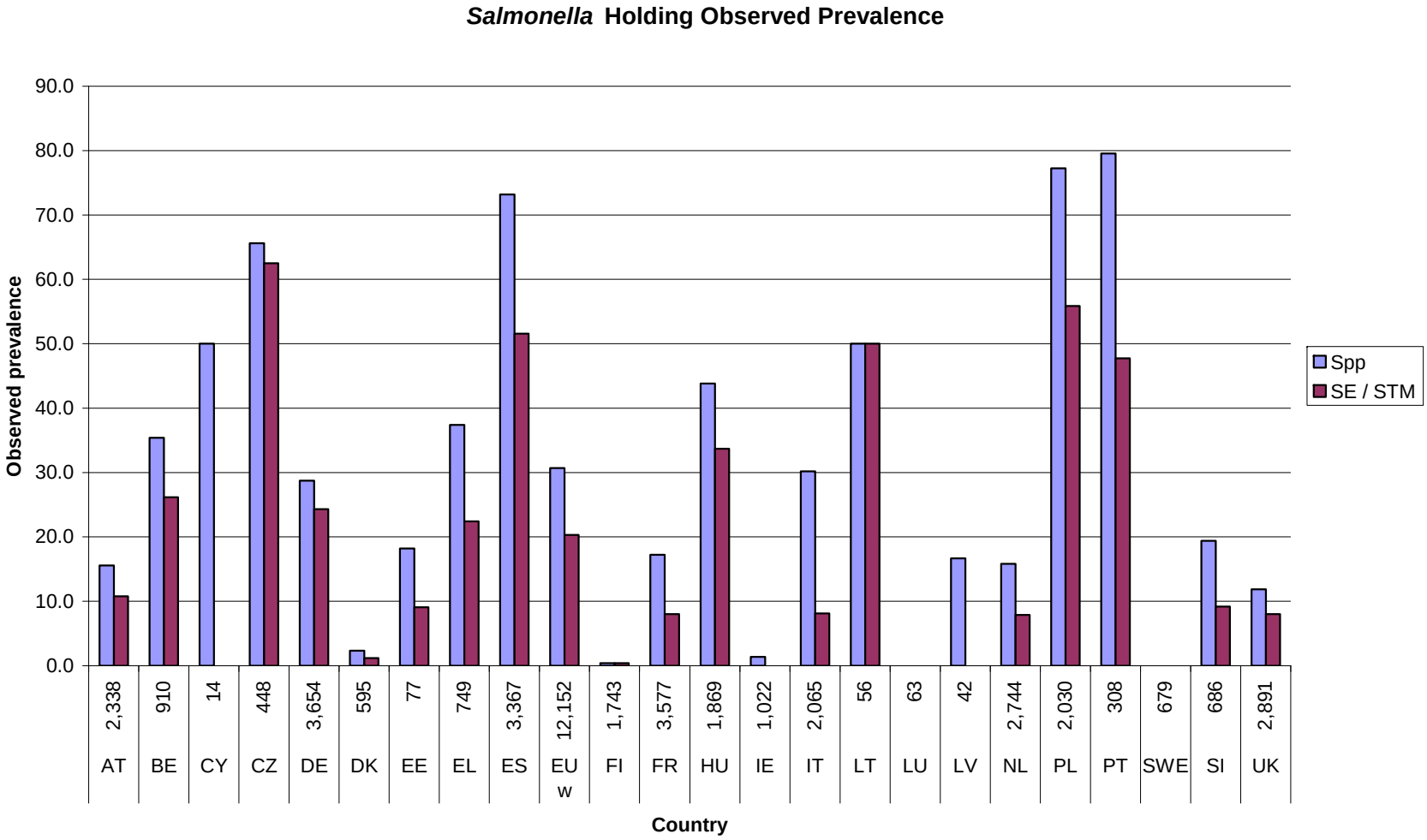


Table 4. ***Salmonella* holding observed prevalence in the EU and Norway, 2004 – 2005 (*full dataset*)**

Member States	Structure-level validated sample (COM)	*S.* spp.				SE/STM			
	N	N	%	95 CI lower limit	95 CI upper limit	N	%	95 CI lower limit	95 CI upper limit
AT	349	56	16.0	*13.4*	*19.1*	37	10.6	*8.4*	*13.2*
BE	150	57	38.0	*32.0*	*44.2*	41	27.3	*22.0*	*33.2*
CY	25	7	28.0	*21.7*	*33.0*	2	8.0	*3.7*	*12.3*
CZ	70	47	67.1	*67.1*	*67.1*	45	64.3	*64.3*	*64.3*
DE	564	166	29.4	*26.2*	*32.8*	140	24.8	*21.8*	*28.1*
DK	161	2	1.2	*0.4*	*2.9*	1	0.6	*0.0*	*2.1*
EE	11	2	18.2	*18.2*	*18.2*	1	9.1	*9.1*	*9.1*
EL	163	89	54.6	*48.8*	*59.9*	49	30.1	*25.1*	*35.4*
ES	507	371	73.2	*70.2*	*75.8*	262	51.7	*48.4*	*54.8*
FI	268	1	0.4	*0.0*	*1.4*	1	0.4	*0.0*	*1.4*
FR	524	93	17.7	*15.1*	*20.7*	41	7.8	*6.1*	*10.0*
HU	277	121	43.7	*39.9*	*47.3*	93	33.6	*30.1*	*37.1*
IE	156	4	2.6	*1.7*	*3.7*	0	0.0	*0.0*	*0.5*
IT	381	113	29.7	*26.0*	*33.5*	30	7.9	*5.9*	*10.4*
LT	11	5	45.5	*26.5*	*58.8*	4	36.4	*19.2*	*51.5*
LU	9	0	0.0	*0.0*	*0.0*	0	0.0	*0.0*	*0.0*
LV	6	1	16.7	*1.0*	*46.8*	0	0.0	*0.0*	*29.1*
NL	471	76	16.1	*13.5*	*19.1*	39	8.3	*6.4*	*10.6*
PL	362	281	77.6	*73.8*	*81.0*	202	55.8	*51.4*	*60.0*
PT	86	61	70.9	*62.6*	*77.5*	35	40.7	*32.8*	*48.7*
SWE	171	0	0.0	*0.0*	*1.3*	0	0.0	*0.0*	*1.3*
SI	108	22	20.4	*16.9*	*24.0*	12	11.1	*8.5*	*14.1*
SK	33	0	0.0	*0.0*	*2.5*	0	0.0	*0.0*	*2.5*
UK	454	54	11.9	*9.7*	*14.4*	36	7.9	*6.2*	*10.1*
EU[3]	5,317	1,629	30.6			1,071	20.1		
EU weighted prevalence			**31.1**	***30.1***	***32.0***		**20.4**	***19.5***	***21.2***
NO	314	0	0.0	*0.00*	*0.0*	0	0.0	*0.00*	*0.0*

[1]: Based on:
a)Technical specifications 'Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU'
Working document Sanco/34/2004 rev3, presented at the meeting of the Standing Committee on the Food Chain and Animal Health 15 July 2004
b) Upgraded data from Member States' final reports

[2]: Validated sampled proportion = actually sampled and validated by EFSA / Total * 100
In the following countries a small proportion of holdings were of size less than 1000 laying hens: CZ, IE, LU, SI

[3]: These EU figures do not include data for MT

Figure 2. ***Salmonella* spp. holding observed prevalence 95% confidence intervals, for EU Member States, for the EU and for Norway, 2004 – 2005 (*clean dataset*)**

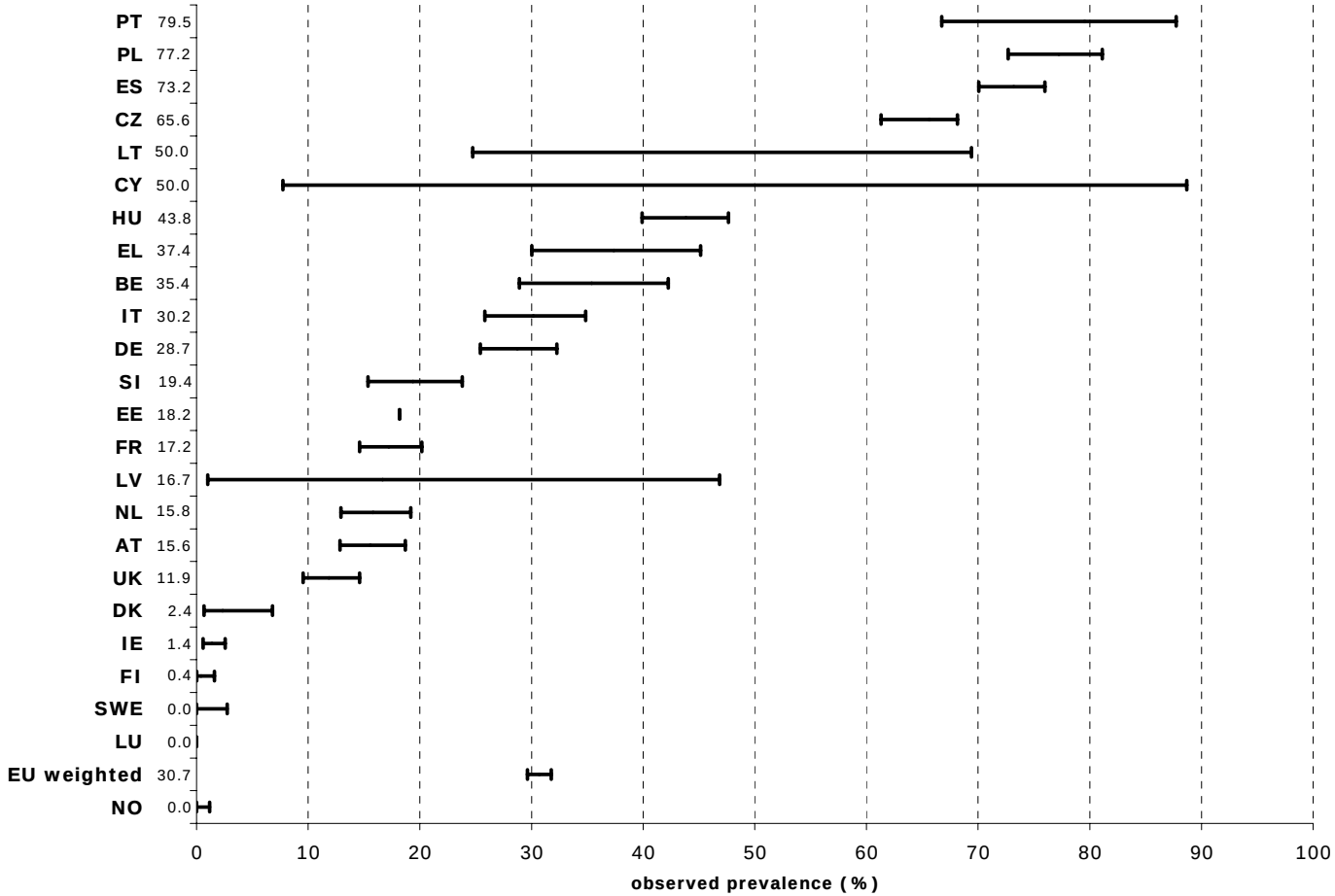
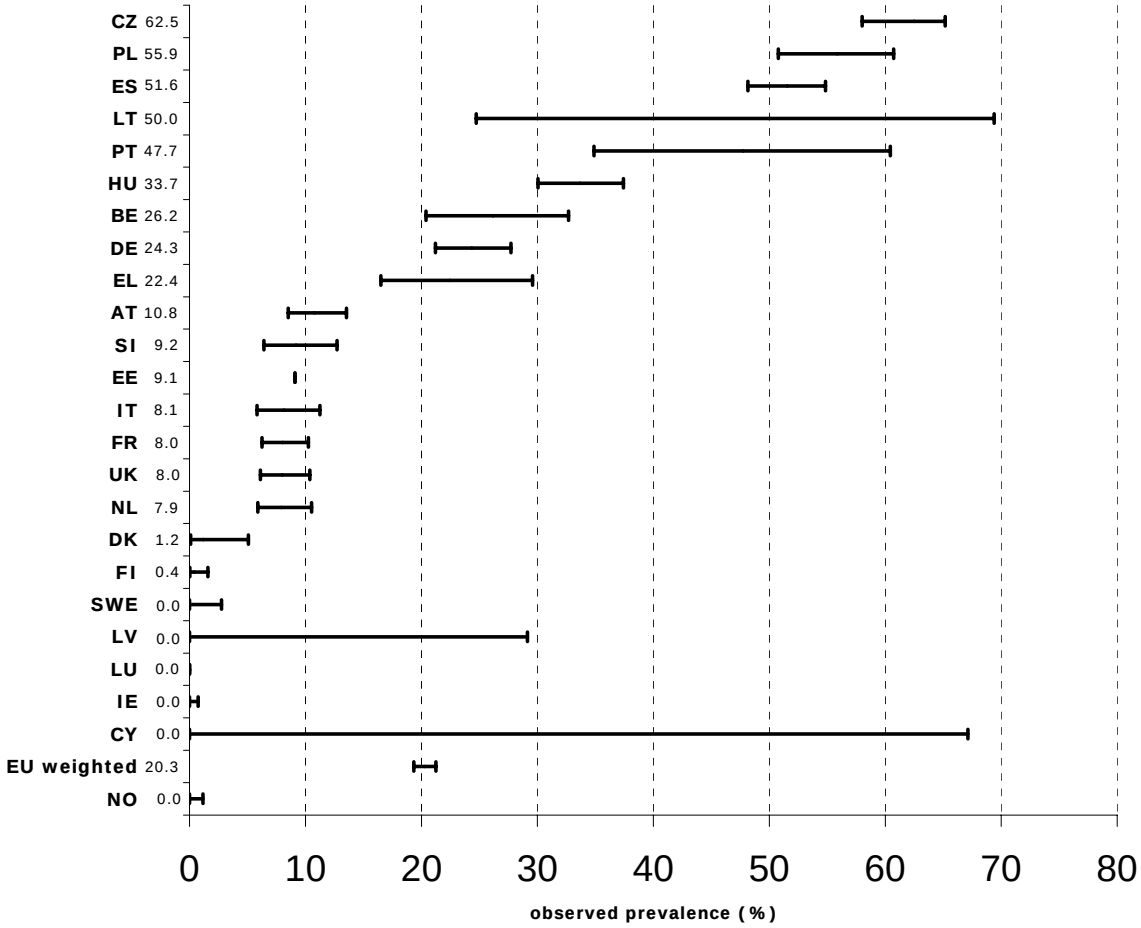


Figure 3. ***Salmonella* Enteritidis / Typhimurium holding observed prevalence 95% confidence intervals, for EU Member States, for the EU and for Norway, 2004 – 2005 (*clean dataset*)**



4.3.2. *Salmonella* within-flock proportion positive samples

Together seven samples were taken from each flock sampled. In positive flocks one to seven samples out of these could have been positive.

The median within-flock proportion positive samples, for *Salmonella* spp. and for SE/STM is presented in Table 5. At the overall EU-level, the median *Salmonella* spp. within-flock proportion positive samples was 57%, while for SE/STM it was 43%. For this latter outcome variable AT, BE, PL, SI and UK had a median within-flock proportion positive samples of at least 50% in the positive flocks, whereas for CZ, DE, DK, EE, EL, ES, FI, FR, HU, IT, LT, NL, and PT this median was below 50%.

The frequency distribution of the within-flock number SE/STM positive samples for positive flocks in the EU and Norway are shown in Figure 4.

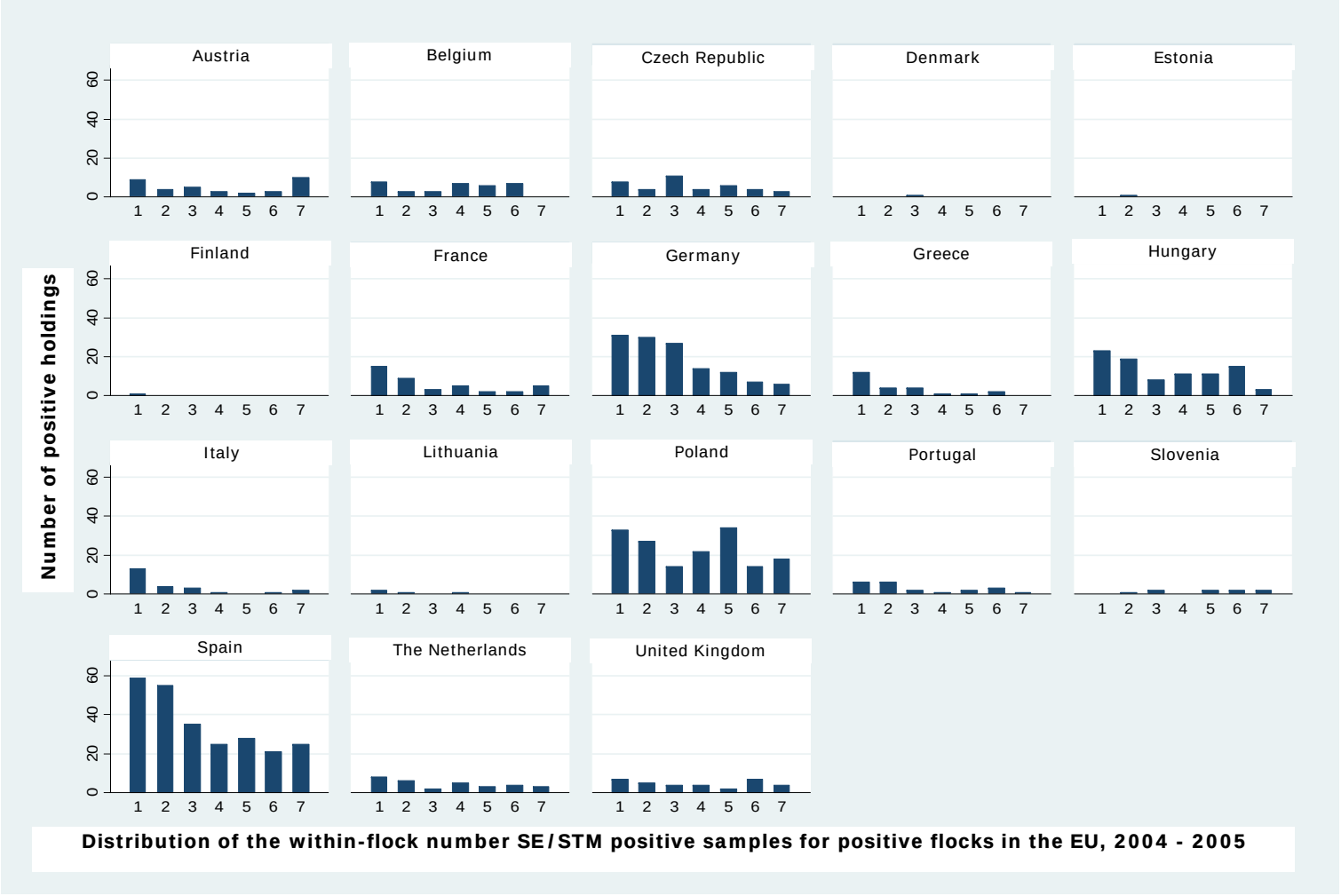
Table 5. **Median within-flock proportion *Salmonella* positive samples in positive flocks observed in the EU and Norway, 2004 – 2005 (*clean dataset*)**

Member States	*S* spp.	SE / STM
AT	0.43	0.50
BE	0.57	0.57
CY	0.57	-
CZ	0.43	0.43
DE	0.43	0.43
DK	0.50	0.43
EE	0.21	0.29
EL	0.29	0.21
ES	0.71	0.43
FI	0.14	0.14
FR	0.29	0.29
HU	0.43	0.43
IE	0.14	- [1]
IT	0.43	0.14
LT	0.21	0.21
LV	0.14	-
NL	0.36	0.43
PL	0.71	0.57
PT	0.57	0.29
SI	0.43	0.71
UK	0.43	0.57
EU [2]	0.57	- 0.43

[1]: '-': no holdings with positive samples

[2]: These EU figures do not include data for MT, SK, LU and SWE: in the latter three countries no Salmonella was isolated

Figure 4. **Graphical display of the within-flock number of SE/STM positive samples, in positive holdings by a fixed within-flock sample size of seven, in the EU and Norway, 2004 – 2005 (*clean dataset*)**



4.4. Frequency of isolated *Salmonella* serovars

The 20 most frequently isolated serovars are listed in Table 6, for the EU and per MS.

Table 6. **Twenty most frequently isolated *Salmonella* serovars, in the EU, 2004 – 2005, and the number of MS where it was isolated**

Name	Number of Isolates	Count of MS that isolate it	Percentage
S. Enteritidis	3340	18	51.33
S. Infantis	541	14	8.31
S. Typhimurium	341	14	5.24
S. Mbandaka	289	12	4.44
S. Livingstone	179	10	2.75
S. Virchow	161	10	2.47
S. Hadar	130	7	2.00
S. Ohio	113	2	1.74
S.Subspec. I Rauform	105	1	1.61
S. Braenderup	99	10	1.52
S. Montevideo	90	9	1.38
S. Agona	77	12	1.18
S. Tennessee	73	9	1.12
other specify	64	3	0.98
S. Bredeney	64	5	0.98
S. Anatum	42	4	0.65
S. Senftenberg	39	9	0.60
S. Newport	35	7	0.54
S. Kentucky	31	4	0.48
S. Indiana	28	4	0.43
S. Rissen	28	10	0.43

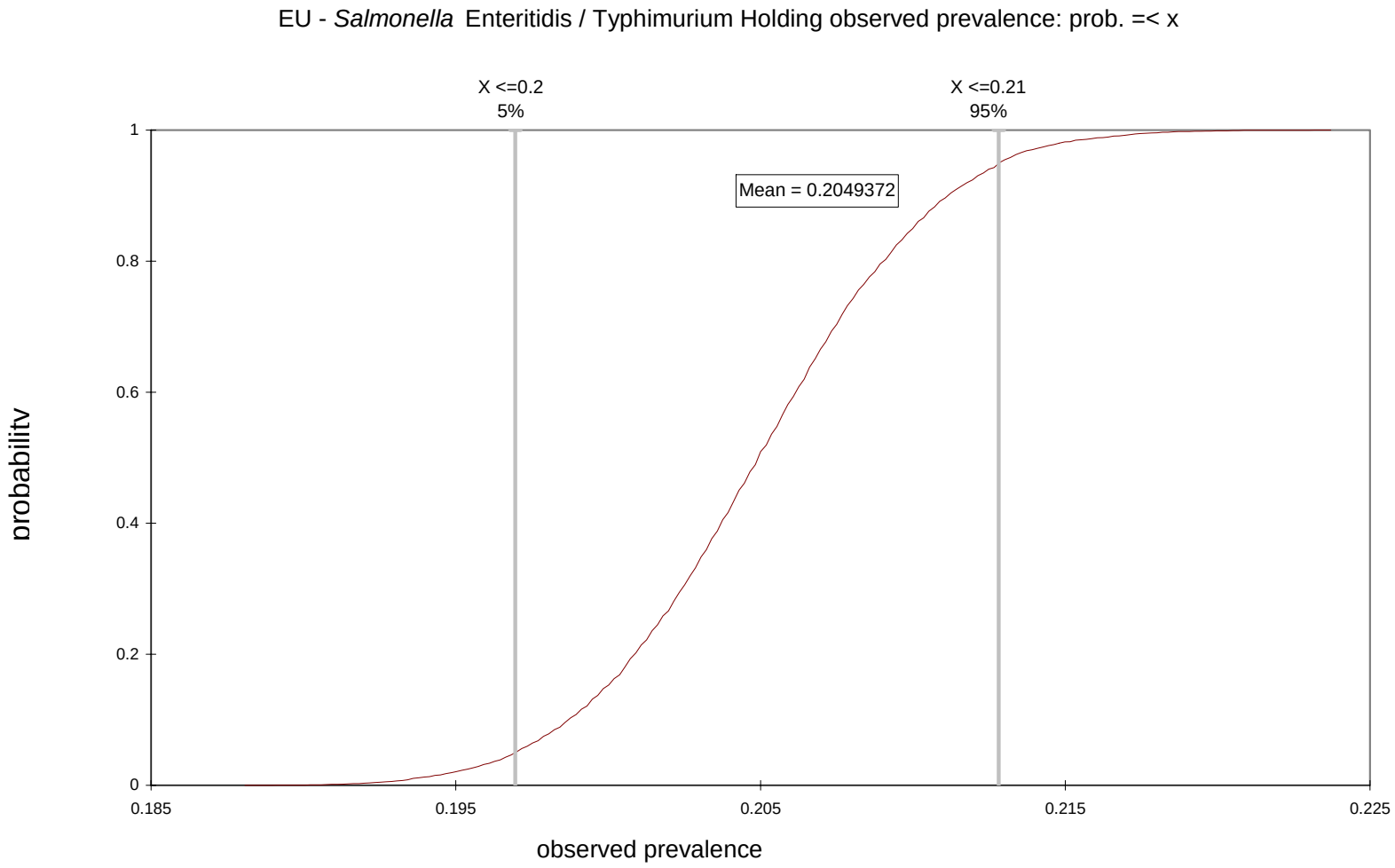
The list of other most frequently isolated serovars is listed in Annex XI, per MS.

4.5. Maximum SE/STM holding observed prevalence

In figure 12 the probability of different maximum observed EU weighted prevalence is presented based on the clean dataset. The EU weighted observed prevalence is with great probability between 19 and 22%.

MS-specific graphs are in the Annex XII in Tables 12 to 33.

Figure 5. **Probability graph: SE or STM holding observed prevalence in the EU =< x, 2004 – 2005 (*clean dataset*)**



4.6. Analysis of factors associated with SE/STM positive samples

The analysis of factors related to the SE/STM sample positivity was a second objective of this study. The recorded factors were chosen to inform about future sampling methodologies, whilst others are potential risk factors.

The analysis of the association of these factors with the SE/STM sample positivity needs to be implemented in a combined way for all factors together in a multiple regression analysis. In a first step of such multiple regression analysis the association of these factors with the SE/STM sample positivity is investigated in a singular way for each factor separately. This is called a univariate analysis. Such a first step can give indications for the multiple regression analysis. But, the results of this univariate analyses must be looked at with caution, since other factors might be affecting the observed positivity for certain stratification.

The following univariate analyses are only presented on a preliminary basis, and are based on the clean dataset that takes account of the 24 countries (all MS - except MT and SK- and NO) and 4,797 holdings.

4.6.1. Impact of vaccination against SE

The factors related to vaccination against SE are important in order to evaluate its possible effect on the within-flock proportion *S.* Enteritidis positive samples.

In this analysis the results from countries having both SE unvaccinated and vaccinated holdings were used. There were eight countries (AT, BE, CZ, EL, ES, FR, IT and PL) where vaccination against SE is neither prohibited nor mandatory, and where between 10-90% of the sampled holdings comprised in the clean dataset declared having used a vaccine against SE.

The following univariate analysis was carried out on this subset of data of the clean dataset (15,484 samples out of 2,212 holdings).

The design-based analysis takes account of the 8 countries and 2,212 holdings.

	Number of samples	*S.* Enteritidis positive samples		
	N	N	(%)	[95% Confidence interval]
flocks unvaccinated against SE	13,979	1,799	(12.9)	[11.8 – 13.9]
flock vaccinated against SE	1,505	60	(4.0)	[1.9 – 6.1]

The *S.* Enteritidis proportion positive sample is higher in flocks unvaccinated against SE, compared to flocks vaccinated against SE.

The same univariate analysis was carried out on a smaller subset of data, notably on *S.* Enteritidis positive holdings. In this way a first exploration is done of the possible impact of vaccination against SE on the within-flock proportion *S.* Enteritidis positive samples, in *S.* Enteritidis positive holdings.

The design-based analysis takes account of the 8 countries and 544 *S.* Enteritidis positive holdings (3,808 samples).

	Number of samples	*S.* Enteritidis positive samples		
	N	N	(%)	[95% Confidence interval]
flocks unvaccinated against SE	3,682	1,799	(48.9)	[46.4 – 51.3]
flock vaccinated against SE	126	60	(47.6)	[33.6 – 61.7]

The *S.* Enteritidis proportion positive sample in *S.* Enteritidis positive holdings seems to be the same in flocks unvaccinated against SE and in flocks vaccinated against SE.

4.6.2. Impact of medication with antimicrobials

The design-based analysis takes account of the 24 countries included in the clean dataset and 4,797 holdings.

The association between SE/STM positivity and medication with antimicrobials during the last two weeks is investigated.

The outcome variable is SE/STM.

	Number of samples	*S.* Enteritidis / Typhimurium positive samples		
	N	N	(%)	[95% Confidence interval]
flocks where no antimicrobials were used during the last two weeks	33,236	3,034	(9.1)	[8.6 – 9.7]
flocks where antimicrobials were used during the last two weeks	343	28	(8.2)	[2.4 – 14.0]

There is no statistical significant difference between the *S.* Enteritidis / Typhimurium proportion positive samples in flocks where no antimicrobials were used during the last two weeks, compared to flocks where there were antimicrobials used during the last two weeks.

4.6.3. Impact of sample type

In the baseline study, barn or free-range laying houses were sampled;
- via faeces (droppings) using boot swabs ('socks'), and
- via dust from barn or free-range houses or from egg belts.

and cage flocks were sampled;
- via faeces from droppings belts, or scrapers, or deep pits, and
- via dust from dusty material beneath cages.

The design-based analysis takes account of the 24 countries in the clean dataset and 4,797 holdings.

The association between SE/STM positivity and sample type is investigated.

For this preliminary analysis, two sample groups were combined;

- faeces, comprising the categories boot swab, pooled faeces from dropping belts, pooled faeces from scrapers, pooled faeces from deep pits and pooled faeces, unspecified, and
- dust, comprising the categories dusty material from beneath cages, dust from egg belts and dust from different places in case of barn or free range flocks

	Number of samples	*S.* Enteritidis / Typhimurium positive samples		
	N	N	(%)	[95% Confidence interval]
Faeces samples	24,008	2,062	(8.6)	[8.0 – 9.2]
Dust samples	9,571	1,000	(10.5)	[9.7 – 11.2]

The *S.* Enteritidis / *S.* Typhimurium proportion positive samples is statistically higher for dust samples, compared to faeces samples.

4.7. Overview of the quality of the bacteriological testing

In the technical specifications of the Baseline study it was indicated that at least one isolate from each positive sample had to be serotyped in the National Reference Laboratory for *Salmonella*, following the Kaufmann-White scheme. For quality assurance of the serotyping, a maximum of 16 typable strains and 16 non-typable isolates of the one year study had to be sent to the CRL-*Salmonella*.

The CRL-*Salmonella* reported on the quality of the bacteriological tests carried out by the NRLs.

Of the 286 typable strains only 14 strains were serotyped differently by the CRL-*Salmonella*. This mostly concerned incidental deviations in some NRLs-*Salmonella*, with the exception of NRL Cyprus. For this latter NRL, five isolates were typed differently by the CRL. The majority of all differences between CRL and NRLs were caused by different serotyping of the H-antigens, as found earlier in the CRL-*Salmonella* interlaboratory comparison studies on typing of *Salmonella.* Some NRLs have indicated to have ordered new and/or other sera to further improve their serotyping.
Of the 67 non-typable strains, the CRL-*Salmonella* was able to further identify 44 strains to serovar names. Several NRLs did not send any non-typable strains or only a few isolates. It is not clear whether these NRLs were better able to identify 'difficult' strains or whether these NRLs obtained only samples with good identifiable strains. Some NRLs with 'roughy' strains asked the CRL for further advice for typing these non-typable strains. It is suggested to make this subject a discussion item at the next CRL-*Salmonella* workshop in 2006. It is important to have a common protocol in Europe how to deal with these kinds of non-typable strains.

5. Discussion

5.1. *Salmonella* observed prevalence

This baseline study for *Salmonella* spp. in laying hen population was the first of this kind of survey conducted in the European Union. Some difficulties in sampling as well as in data entry and transfer were encountered. Therefore not all collected data met the technical specifications designed for this study. This resulted in a number of samples and consequently holdings sampled being excluded from the clean dataset, which was mainly used for analyses. Certain Member States with a rather high numbers of holdings excluded ended up with a relevant sample size reduction that widened confidence interval of their holding observed *Salmonella* prevalence. Therefore, the prevalences were also calculated from the full dataset, containing all data on samples that EFSA received.

The EU prevalence figures are presented as weighted means of the prevalences in the Member States, where the national prevalences are weighted by the size of laying hen holding populations in each country.

Using the clean dataset the *Salmonella* spp. EU weighted holding observed prevalence is 30.7%, which means that on average in one of three large-scale laying hen holdings with laying hens at the end of their production period, a *Salmonella* serovar was isolated. The corresponding *Salmonella* spp. prevalence in the full dataset was 31.1%.

As regards *S.* Enteritidis / *S.* Typhimurium - presence of SE or STM or both SE and STM - the EU weighted holding observed prevalence was 20.3% in the clean dataset. This figure was consistent with the figure from the full dataset, 20.4%. The observed prevalence means that on average one in five large-scale laying hen holdings with hens during the last nine weeks of their production, had at least one of the seven samples positive to SE or to STM or to both.

When investigating the MS-specific figures, it became clear that there are important differences between countries, in such a way that an EU weighted mean can be regarded as largely meaningless. Indeed, the variation in the observed *Salmonella* spp. and SE/STM holding observed prevalences for the countries were very large, ranging from 0% to 79.5% and 0% to 62.5%, respectively. It means that all prevalence scenarios are present in the EU: very low to low, moderate, and high to very high.

The comparison between the prevalence figures calculated from the clean and full datasets indicates that there is no systematic exclusion or inclusion of observations from positive flocks. However, for a number of Member States there were differences in the observed holding prevalence figures, but there seemed to be no trend whether the prevalence figures would be higher in the clean compared to the full dataset.

Both the observed prevalences for *Salmonella* spp. and SE/STM in Member States in this study were substantially higher when compared to the prevalences reported by the Member States for laying hen flocks in the national zoonoses reports for 2004[5]. This indicates that the sampling design, e.g. the number and type of samples taken from a flock, were much more sensitive than those used normally by the most Member States.

The holding observed prevalence must be considered as a minimum, as the study sampling design was based on random sampling of one flock per holding. This means that there was an important chance of sampling a negative flock even if the holding had some positive flocks. Therefore the holding prevalence, determined by surveying all flocks, would on average be higher than the flock prevalence. This is the reason why the study results were not adjusted for numbers of flocks per holding.

It should be kept in mind that a flock found positive in this study does not necessarily mean a flock including infected birds, because the samples were collected from the environment of the birds, i.e. faecal droppings and dust. However, these types of environment samples have proved sensitive indicators for *Salmonella* infection in a flock.

Substantial numbers of holdings were positive for SE/STM from one to two samples out of the seven samples taken. This may indicate that reduction of the number of samples taken from a flock might reduce the numbers of flocks found positive. However, direct comparison between different numbers of environmental samples is difficult, because fewer samples are usually collected correspondingly from a larger area.

5.2. Frequency of isolated *Salmonella* serovars

The most frequently isolated *Salmonella* serovar from the laying hen flocks was by far *S.* Enteritidis, which was isolated in 18 countries and accounted for 50.8% of the reported isolates, based on the clean database. *Salmonella* Infantis was the second most encountered isolate (8.3%), isolated in 14 countries, whereas *Salmonella* Typhimurium was the third most frequent isolated one (5.2%) in 14 countries in total. The distribution of the isolated serovars varied between the countries; however, *S.* Enteritidis was the most frequent one in 15 MSs.

5.3. SE/STM reduction targets

Salmonella reduction targets may be absolute or relative. Setting an absolute target becomes more difficult when the variation in the prevalences is large, as was the case in this study. Thus, setting a relative target could be considered. However, if relative targets are to be set (for example, a certain percentage of reduction over observed prevalence), then a minimum accepted prevalence should be laid down for countries having already a very low prevalence. This can be applied by a combination of a 'relative target' and a 'tolerated maximum limit' (of the observed holding prevalence).

The graphs plotting the probabilities of different maximum observed prevalences can be used as a tool to investigate *Salmonella* reduction target settings.

5.4. Preliminary analysis of factors associated with *Salmonella* positivity

Five faeces samples and two dust samples were taken from each flock. In eight countries where both flocks vaccinated and unvaccinated against SE were sampled, holdings having SE vaccinated flocks were less likely to be positive for SE. But with the subgroup of SE positive holdings in these countries, there was no difference in the proportion SE positive samples between SE vaccinated and unvaccinated flocks.

Dust samples were found to be more positive to SE/STM compared to faeces samples, indicating the higher relative sensitivity of dust samples. Medication with antimicrobials during the last two weeks before sampling did not seem to have an impact on the SE/STM sample proportion positive samples. However, further weighting these factors in a combined multiple regression analysis is essential.

5.5. Relevance of the findings to human salmonellosis

Overall, in the EU *S.* Enteritidis and *S.* Typhimurium are the *Salmonella* serovars most frequently associated with human illness[5]. Eggs are considered the predominant source of human salmonellosis in Europe as well as many other countries worldwide[6].

For the likelihood of eggs being contaminated with *Salmonella* depends amongst others on the flock prevalence, within-flock prevalence, and the frequency that infected hens lay contaminated eggs. In naturally infected layer flocks the proportion of infected eggs that are laid varies[7]. However, many studies show this proportion to be mostly below 3%[8,9,10,11,12,13].

The *Salmonella* observed holding prevalence in flocks of laying hens in this study underlines the need for controlling *Salmonella* in the table-egg production sector.

6. Concluding remarks

This baseline study has established a baseline holding observed *Salmonella* prevalence, which can be used to set the EU *Salmonella* reduction target. The baseline prevalence figures may be used later to compare future trends and follow the impact of control programmes. The other variables studied, such as the proportion of positive samples in holdings and the sample types, will also help the target setting by defining the verification procedures for meeting the target.

The sampling design used proved to be very sensitive for *Salmonella* detection in laying hen flocks. A large variation in the holding observed prevalence between Member States was observed, which could suggest for setting a relative target for a defined time period.

It proved difficult for Member States to fully comply, in due time, with the technical specifications of this study. For future baseline studies: it is suggested to improve the degree of compliance to technical specifications by a more anticipative coordination and by contents-level software validation tools that check for non-valid data while MSs send in data.

Annexes

Annex I. Overview of the data exclusion criteria grid

In the following section the variables are uniquely identified using the 'item integer' mentioned in the ad hoc Data Dictionary.

In a first step the following records were excluded.

Criterion 1
007 Hens in holding: =< 30
This criterion excludes all records containing less than 30 hens in the holding.

Criterion 2
007 Hens in holding: < values for 011 Number of hens in flock
This criterion excludes all records containing a number of hens in the holding that is smaller than the number of hens in the flock. A 10% difference (in case '007 hens in holding' is a smaller number compared to '011 Number of hens in flocks') was allowed.

Criterion 3
008 Number of flocks: <= 0
This criterion excludes all records containing a number of flocks equal to, or lower than, zero.

Criterion 4
008 Number of flocks: > 20
This criterion excludes all records containing a number of flocks higher than 20.

Criterion 5
008 Number of flocks: values: = 1 and value for 007 Hens in holding IS NOT EQUAL TO value for 011 Number of hens in flock
This criterion excludes all records with one flock in the holding where the number of hens in that holding does not equal the number of hens in the flock. A 10% difference in either direction was allowed for.

Criterion 6
010 Date of sampling: < 15 September 2004
This criterion excludes all records containing a date of sampling before 15 September 2004.

Criterion 7
010 Date of sampling: > 15 October 2005
This criterion excludes all records containing a date of sampling after 15 October 2005.

Criterion 8
011 Number of hens in flock: < 30
This criterion excludes all records containing flocks with less than 30 hens.

Criterion 9
013 Age of hens at sampling: > 150
This criterion excludes all records containing hens aged more than 150 weeks.

Criterion 10
013 Age of hens at sampling: < 30 and 014 Maximum age of hens at sampling: IS NUL
This criterion excludes all records containing hens aged less than 30 weeks in homogeneous age flocks.

Criterion 11
013 Age of hens at sampling: <= 0
This criterion excludes all records containing hens aged zero weeks or less.

Criterion 12
014 Maximum age of hens at sampling: > 150
This criterion excludes all records containing hens aged more than 150 weeks in mixed age flocks.

Criterion 13
014 Maximum age of hens at sampling: EQUAL TO 013 Age of hens at sampling
This criterion excludes all records containing hens in mixed age flocks where the minimum and maximum age is the same.

Criterion 14
015 Expected depopulation date: < 1 October 2004
This criterion excludes all records containing an expected depopulation date before 1 October 2004.

Criterion 15
015 Expected depopulation date: > 30 June 2007
This criterion excludes all records containing an expected depopulation date after 30 June 2007.

Criterion 16
015 Expected depopulation date: < value of 010 Date of sampling
This criterion excludes all records containing an expected depopulation before the date of sampling.

Criterion 17a
016 Expected depopulation date accuracy: no and [difference between (015 Expected depopulation date and 010 Date of sampling) > 63] and 011 Number of hens in flocks: >= 1000 and 014 Maximum age of hens at sampling: < 60
This criterion excludes all records containing flocks of mixed age sized 1000 hens or more, with an accurate expected depopulation date, where hens are not sampled within a maximum of 9 weeks (63 days) before depopulation, and where the maximum age of hens was below the age of 60 weeks.

Criterion 17b
016 Expected depopulation date accuracy: no and [difference between (015 Expected depopulation date and 010 Date of sampling) > 63] and 011 Number of hens in flocks: >= 1000 and 013 Age of hens at sampling: < 60 and 014 Maximum age of hens at sampling: IS NULL
This criterion excludes all records containing flocks of homogeneous age and sized 1000 hens or more, with an accurate expected depopulation date, where hens are not sampled within a maximum of 9 weeks (63 days) before depopulation, and where hens were below the age of 60 weeks.

Criterion 18
016 Expected depopulation date accuracy: no and [difference between (015 Expected depopulation date and 010 Date of sampling) > 63] and 011 Number of hens in flocks: < 1000 and 013 Age of hens at sampling: < 60 and 014 Maximum age of hens at sampling: IS NULL
This criterion excludes all records containing flocks of homogeneous age and sized less than 1000 hens, with an accurate expected depopulation date, where hens are not sampled within a maximum of 9 weeks (63 days) before depopulation, and where hens were below the age of 60 weeks.

Criterion 19

017 Vaccination status: no and 018 Vaccination type: IS NOT NULL

This criterion excludes all records containing unvaccinated flocks with information of the type of vaccination.

Criterion 20

017 Vaccination status: unknown and 018 Vaccination type: IS NOT NULL

This criterion excludes all records containing flocks with an unknown vaccination status with information of the type of vaccination.

Criterion 21

017 Vaccination status: no and 019 Vaccination period: IS NOT NULL

This criterion excludes all records containing unvaccinated flocks with information of the vaccination period.

Criterion 22

017 Vaccination status: unknown and 019 Vaccination period: IS NOT NULL

This criterion excludes all records containing flocks with an unknown vaccination status with information of the vaccination period.

Criterion 23

017 Vaccination status: no and 020 Vaccination name : IS NOT NULL

This criterion excludes all records containing unvaccinated flocks with information of the vaccination name.

Criterion 24

017 Vaccination status: unknown and 020 Vaccination name : IS NOT NULL

This criterion excludes all records containing flocks with an unknown vaccination status with information of the vaccination name.

Criterion 25

021 Medication status: no and 022 Medication-antimicrobial name IS NOT NULL

This criterion excludes all records containing flocks where no antimicrobials were used during the last two weeks, with information of the antimicrobial name.

Criterion 26

026 Date of bacteriological detection testing: < 15 September

This criterion excludes all records containing a date of primary testing in the laboratory before 15 September 2004.

Criterion 27

026 Date of bacteriological detection testing: > 30 November 2005

This criterion excludes all records containing a date of primary testing in the laboratory after November 2005.

Criterion 28

026 Date of bacteriological detection testing: < value of 010 Date of sampling

This criterion excludes all records containing a date of primary testing in the laboratory before the date of sampling.

Criterion 29

027 Specimen status: no and 028 Specimen characteristics IS NULL and 029 Specimen characteristics comment IS NULL (EMPTY)

This criterion excludes all records containing specimen characteristics non compliant to the technical specifications but with no information in the field 'specimen characteristics' and no information in the field 'specimen characteristic comment'.

Criterion 30

032 Reference of laboratory for serotyping: IS NULL (EMPTY) and 030 Test result is 'positive'

This criterion excludes all records containing positive test results without information of the reference laboratory.

Criterion 31

032 Reference of laboratory for serotyping: IS NOT NULL (NOT EMPTY) and **030 Test result is 'negative'**

This criterion excludes all records containing negative test results with information of the reference laboratory.

Criterion 32

033 Isolate (Salmonella serovar): IS NULL (EMPTY) and 030 Test result is 'positive'

This criterion excludes all records containing positive test results with no information of the isolate.

Criterion 33

033 Isolate (Salmonella serovar): IS NOT NULL (NOT EMPTY) and 030 Test result is 'negative'

This criterion excludes all records containing negative test results with information of the isolate.

Criterion 34

014 Maximum age of hens at sampling: < 30

This criterion excludes all records containing hens aged less than 30 weeks in mixed age flocks.

Criterion 35

Difference date between: '010 Date of sampling' and '026 Date of bacteriological detection testing': >= 7

This criterion excludes all records containing a 'days to bacteriological start of test' above 7 days.

In a second step holdings were excluded with less than 7 samples, which were those holdings were at least one sample did not meet one of the aforementioned exclusion criteria.

Annex II. Overview of the sampled holdings (*full dataset*)

Member States	Number of holdings having at least 1000 laying hens				
	Total[1]	To be sampled[1]	Actually sampled	Actually sampled and validated by COM and sent to EFSA	
			N	N	% [2]
AT	769	362	361	349	45.4
BE	395	251	149	150	38.0
CY	28	27		25	89.3
CZ	70	70	70	70	100.0
DE	2,419	533	563	564	23.3
DK	263	190	190	161	61.2
EE	11	11	11	11	100.0
EL	352	232	?	163	46.3
ES	1,100	422		507	46.1
FI	558	307	268	268	48.0
FR	1,840	518	524	524	28.5
HU	464	276		277	59.7
IE	180	172	165	156	86.7
IT	1,168	428	346	381	32.6
LT	17	17		11	64.7
LU	9	9		9	100.0
LV	16	16	18	6	37.5
NL	1,553	474	474	471	30.3
PL	1,238	440	440	362	29.2
PT	220	166		86	39.1
SWE	351	210	167	171	48.7
SI	138	138	110	108	78.3
SK	39	39	36	33	84.6
UK	1,202	436	454	454	37.8
EU[3]	14,400	5,744	4,346	5,317	36.9
NO	761	360	318	314	41.3

[1]: Based on Technical specifications 'Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU'. In case more updated figures have been provided in the Member States' final reports on the study results, this figure has been used.

[2]: Validated sampled proportion = actually sampled and validated by COM / Total * 100
In the following countries a small proportion of holdings were of size less than 1000 laying hens: CZ, IE, LU, SI

3: These EU figures do not include data for MT

Annex III. Overview of the sampled holdings (*clean dataset*)

Member States	Number of holdings having at least 1000 laying hens				
	Total[1]	To be sampled[1]	Actually sampled	Actually sampled and validated by EFSA	*Validated sampled proportion*[2]
			N	N	%
AT	769	362	361	334	*43.4*
BE	395	251	149	130	*32.9*
CY	28	27		2	*7.1*
CZ	70	70	70	64	*91.4*
DE	2,419	533	563	522	*21.6*
DK	263	190	190	85	*32.3*
EE	11	11	11	11	*100.0*
EL	352	232	-	107	*30.4*
ES	1,100	422	-	481	*43.7*
FI	558	307	268	249	*44.6*
FR	1,840	518	524	511	*27.8*
HU	464	276	-	267	*57.5*
IE	180	172	165	146	*81.1*
IT	1,168	431	-	295	*25.3*
LT	17	17	-	8	*47.1*
LU	9	9	-	9	*100.0*
LV	16	16	18	6	*37.5*
NL	1,553	474	474	392	*25.2*
PL	1,238	440	440	290	*23.4*
PT	220	166	-	44	*20.0*
SWE	303	210	167	97	*32.0*
SI	138	138	110	98	*71.0*
UK	1,202	436	454	413	*34.4*
EU[3]	14,313	5,708	3,964	4,561	*31.9*
NO	761	360	318	236	*31.0*

[1]: Based on Technical specifications 'Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU'
In case more updated figures have been provided in the Member States' final reports on the study results, this figure has been used.

[2]: Validated sampled proportion = actually sampled and validated by EFSA / Total * 100
In the following countries a small proportion of holdings were of size less than 1000 laying hens: CZ, IE, LU, SI

[3]: These EU figures do not include data for MT and SK

Annex IV. Overview of the samples (*clean dataset*)

Member States	Sample population[1] (number of hens)	Samples validated by EFSA
		N
AT	1,031,028	2,338
BE	3,372,306	910
CY	12,200	14
CZ	4,583,871	448
DE	9,457,938	3,654
DK	1,226,378	595
EE	732,899	77
EL	2,682,635	749
ES	28,960,834	3,367
FI	1,425,350	1,743
FR	6,151,583	3,577
HU	5,013,659	1,869
IE	748,170	1,022
IT	10,644,374	2,065
LT	345,197	56
LU	1,156,163	63
LV	55,920	42
NL	9,664,363	2,744
PL	10,523,273	2,030
PT	3,601,107	308
SWE	1,491,063	679
SI	544,552	686
UK	11,354,338	2,891
EU[2]	114,779,201	31,927
Norway	1,030,692	1,652

[1]: Sum of the holding sizes (hens present in the holdings at the time of sampling)

[2]: These EU figures do not include data for MT and SK

Annex V. General features of the European laying hens population

An overview of the European laying hen holdings and laying hen population is given in Table 7 and Table 8. These figures are reported by EUROSTAT. EUROSTAT tables have the advantage of being exhaustive and complete.
Both tables are ranked in descending order by the size of the subpopulation – being the number of holdings and birds, respectively - within the category 'holding size above 1.000'.
In the EU there were in 2003 – the new MS included – 17,400 laying hen holdings with a holding size of at least 1,000 laying hens. France has the highest number of laying hen holdings (3,190), whereas Latvia and Estonia have the smallest number (10). In the subpopulation 'holding size above 1.000' there were approximately 411 million laying hens. France has the highest number of laying hens (71.52 million laying hens), whereas Slovenia have the smallest number (350,000).

In addition, the density of the laying hen holding and laying hens population in the MS was determined by dividing the number of laying hen holdings and laying hens, within the aforementioned EUROSTAT category 'holding size above 1.000', by the amount of utilized agricultural area in km^2. Data on the amount of agricultural land per MS were obtained from Eurostat.

Figure 6 displays the laying hen holding density. MT, CY, NL and AT are those MS were the density of laying hen holdings is highest, respectively 73, 10, 7 and 6 laying hen holdings per km^2. It is lowest in SK and LT, respectively .44 and .35.

Figure 7 displays the laying hen density. In MT and NL the density of laying hens is highest, respectively 4,477 and 1,929 laying hens per km^2. It is lowest in Ireland, 46.

Only very few data regarding the number of laying hens flocks in the EU or its MSs are available. Moreover, the few available data are not comparable regarding the year they pertain to.

Table 7. **Laying hen holding population in the EU and Norway, 2003**

Holdings with laying hens by holding size classes, and ranked by the number of holdings with size above 1.000

YEAR 2003

	number of holdings									
	total	**1 - 99**	**100 - 999**	**1.000 - 2.999**	**3.000 - 4.999**	**5.000 - 9.999**	**10.000 - 29.999**	**>= 30.000**		***>= 1.000*** [1]
FR	162,690	158,000	1,500	360	450	860	880	640		*3,190*
DE	88,340	81,060	4,610	1,160	380	350	450	330		*2,670*
PL	997,390	989,990	5,460	480	270	470	430	290		*1,940*
UK	33,420	30,050	1,590	320	210	380	580	300		*1,790*
ES	184,710	182,530	690	170	70	310	520	410		*1,480*
NL	1,680	260	180	80	80	200	540	350		*1,250*
AT	64,630	62,600	1,190	460	160	130	80	20		*850*
IT	128,680	126,740	1,210	40	50	50	320	270		*730*
FI	1,890	960	260	310	130	120	90	20		*670*
BE	5,020	4,310	130	50	40	120	200	160		*570*
EL	323,620	320,330	2,820	200	70	110	70	30		*480*
HU	410,200	408,710	1,100	190	50	60	50	50		*400*
SWE	5,530	5,030	170	60	40	90	100	50		*340*
DK	3,810	3,410	140	20	40	60	90	40		*250*
IE	7,480	7,200	80	50	30	70	30	20		*200*
PT	204,760	204,340	240	0	30	10	60	80		*180*
CZ	21,950	21,690	110	40	10	10	30	60		*150*
SI	47,890	47,620	190	40	10	20	10	0		*80*
MT	920	840	10	30	10	10	20	-		*70*
SK	46,440	46,330	50	10	0	0	10	30		*50*
LT	185,850	185,760	70	10	0	-	0	10		*20*
CY	8,740	8,610	100	0	0	0	10	10		*20*
LV	66,200	66,100	90	0	-	-	0	10		*10*
EE	15,070	14,900	150	-	0	0	0	10		*10*
LU	660	630	30	0	0	0	0	-		-
EU total	3,017,570	2,978,000	22,170	4,080	2,130	3,430	4,570	3,190	0	*17,400*
NO	3,080	-	-	-	-	-	-	-		-

[1]: at least

-: no data available

Figure 6. **Laying hen holding density in the EU and Norway, 2003**

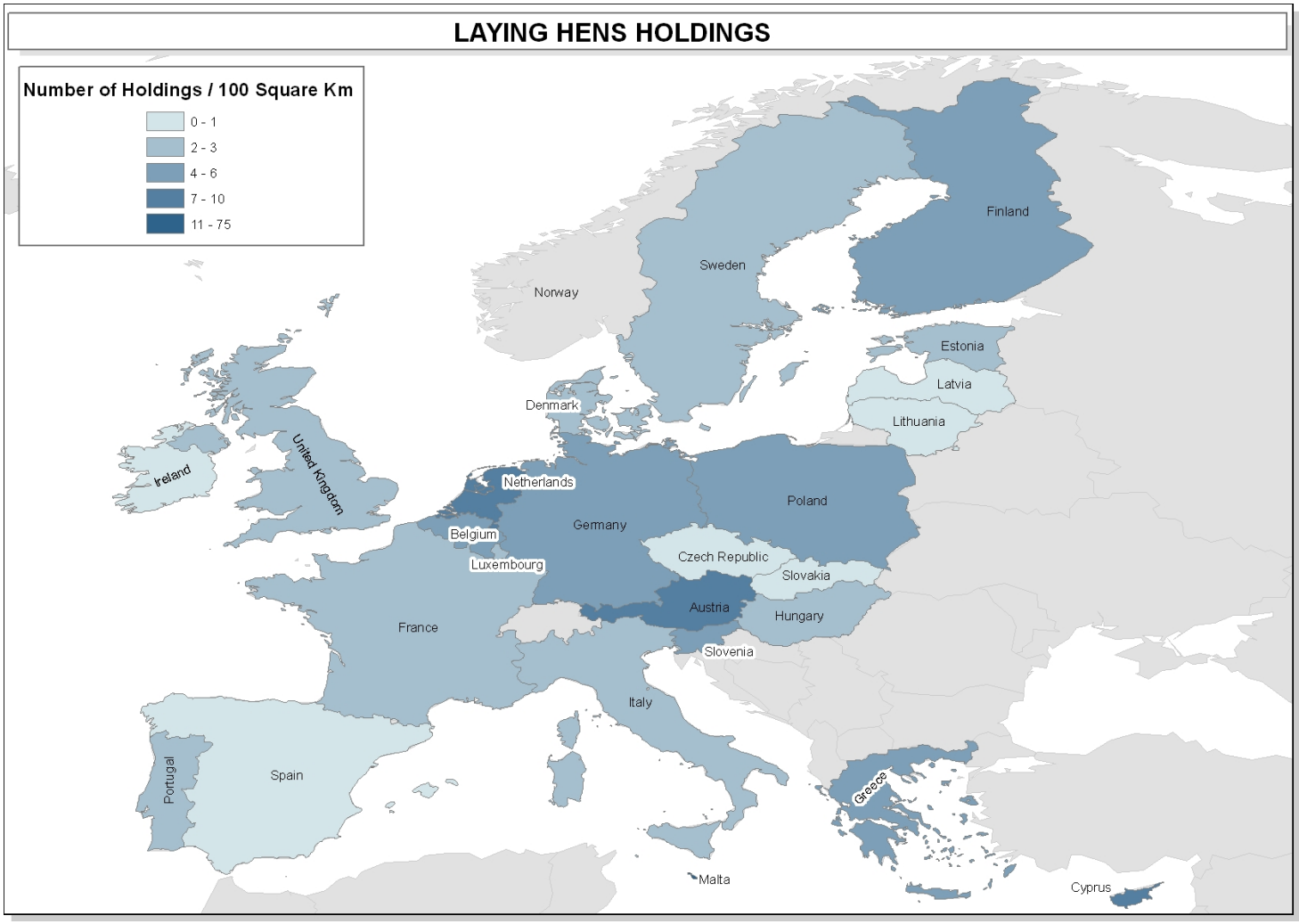


Table 8. Laying hens population in the EU and Norway, 2003

Laying hens by holding size classes, and ranked by the number of laying hen subpopulation size within holding size above 1.000

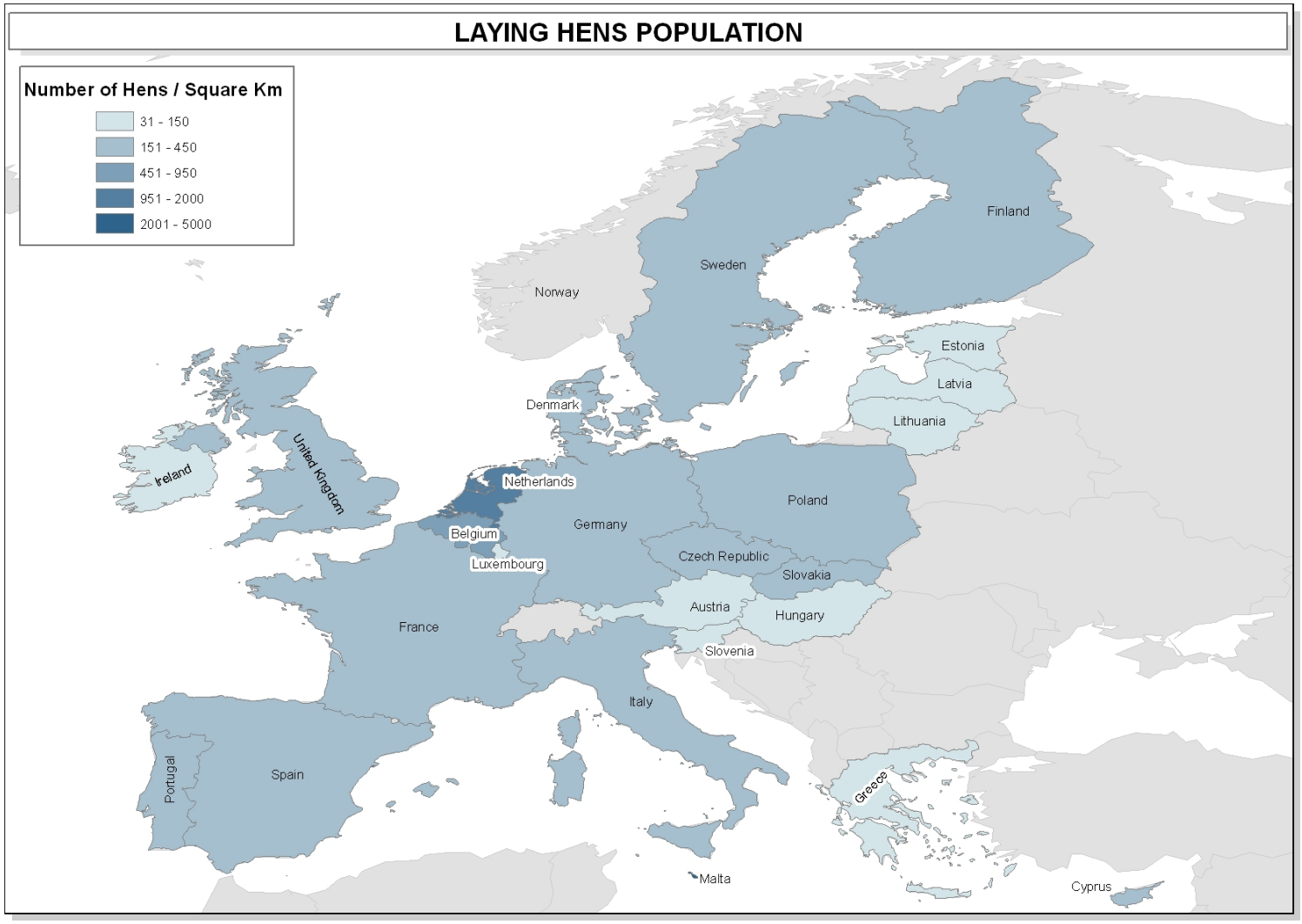
YEAR 2003

	number of laying hens								
	total	**1 - 99**	**100 - 999**	**1.000 - 2.999**	**3.000 - 4.999**	**5.000 - 9.999**	**10.000 - 29.999**	**>= 30.000**	***>= 1.000*** [1]
FR	73,850,000	1,930,000	400,000	660,000	1,770,000	6,130,000	13,870,000	49,090,000	*71,520,000*
ES	59,510,000	2,100,000	130,000	250,000	260,000	2,130,000	8,890,000	45,750,000	*57,280,000*
DE	55,690,000	1,520,000	1,430,000	1,990,000	1,450,000	2,430,000	8,060,000	38,820,000	*52,750,000*
UK	48,330,000	490,000	440,000	590,000	830,000	2,670,000	9,880,000	33,430,000	*47,400,000*
NL	36,950,000	10,000	50,000	150,000	300,000	1,450,000	9,570,000	25,430,000	*36,900,000*
PL	51,760,000	15,390,000	990,000	760,000	980,000	3,160,000	7,110,000	23,360,000	*35,370,000*
IT	35,390,000	1,570,000	310,000	80,000	190,000	390,000	5,580,000	27,280,000	*33,520,000*
BE	13,070,000	60,000	30,000	100,000	180,000	900,000	3,320,000	8,480,000	*12,980,000*
CZ	10,490,000	300,000	30,000	60,000	40,000	90,000	500,000	9,460,000	*10,150,000*
PT	11,530,000	1,850,000	30,000	0	100,000	70,000	1,020,000	8,450,000	*9,640,000*
HU	14,360,000	7,210,000	270,000	320,000	190,000	400,000	810,000	5,150,000	*6,870,000*
SWE	6,010,000	90,000	40,000	110,000	170,000	600,000	1,780,000	3,220,000	*5,880,000*
EL	11,380,000	6,130,000	390,000	310,000	250,000	670,000	980,000	2,650,000	*4,860,000*
DK	4,900,000	50,000	30,000	30,000	150,000	420,000	1,500,000	2,710,000	*4,810,000*
AT	6,050,000	950,000	400,000	800,000	580,000	900,000	1,270,000	1,150,000	*4,700,000*
FI	4,300,000	20,000	110,000	580,000	500,000	820,000	1,500,000	770,000	*4,170,000*
SK	4,610,000	500,000	10,000	20,000	0	0	240,000	3,790,000	*4,050,000*
LT	4,020,000	1,750,000	10,000	10,000	0	-	0	2,200,000	*2,210,000*
IE	2,110,000	70,000	30,000	100,000	130,000	500,000	490,000	790,000	*2,010,000*
LV	2,550,000	790,000	20,000	0	-	-	0	1,670,000	*1,670,000*
EE	1,230,000	210,000	30,000	-	0	0	0	890,000	*890,000*
CY	760,000	150,000	20,000	0	0	0	230,000	320,000	*550,000*
MT	510,000	10,000	0	50,000	40,000	80,000	320,000	-	*490,000*
SI	1,390,000	550,000	50,000	60,000	50,000	140,000	100,000	0	*350,000*
LU	60,000	10,000	10,000	0	0	0	0	-	-
EU total	460,810,000	43,710,000	5,260,000	7,030,000	8,160,000	23,950,000	77,020,000	294,860,000	*411,020,000*
NO	4,470,000	-	-	-	-	-	-	-	-

[1]: at least

-: no data available

Figure 7. **Laying hen density in the EU and Norway, 2003**



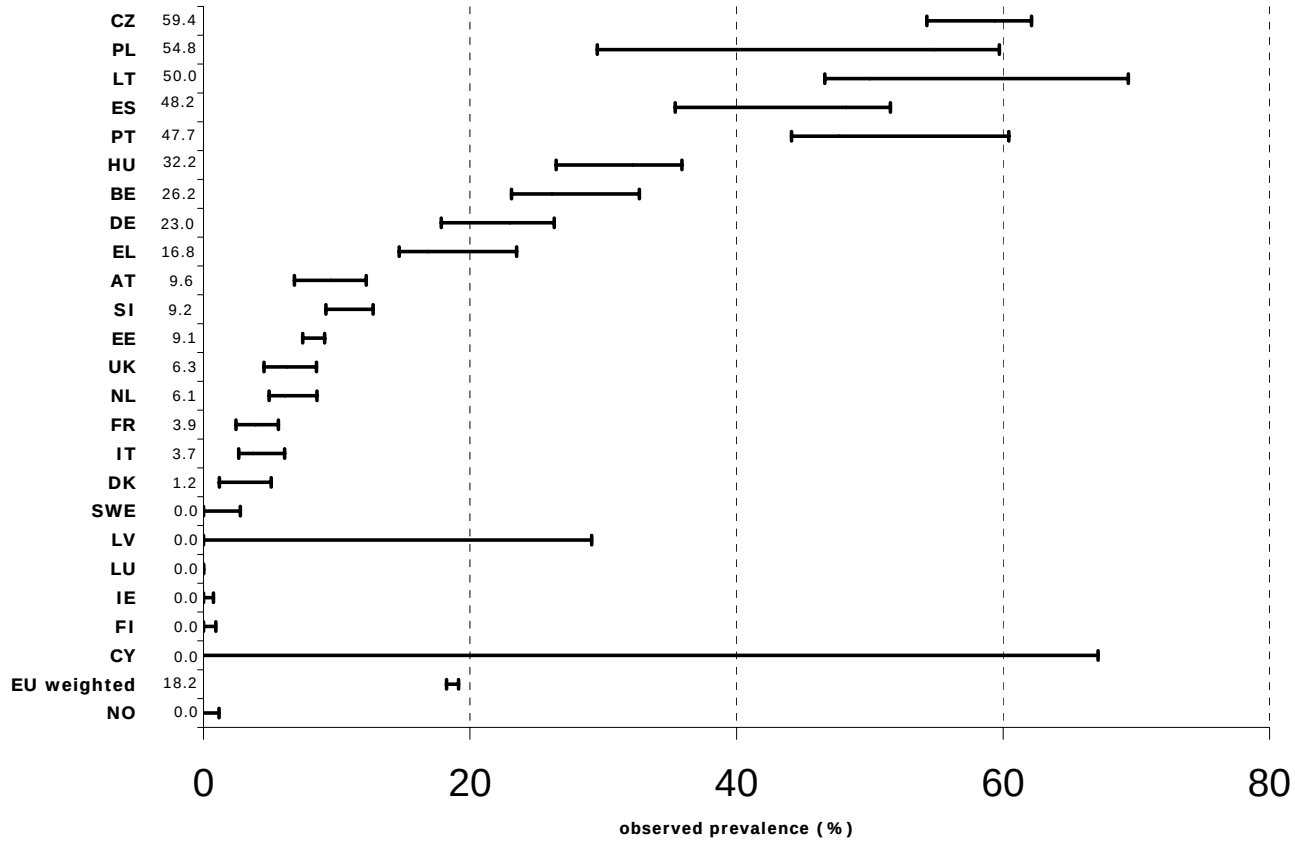
Annex VI. *Salmonella* Enteritidis and *Salmonella* Typhimurium holding observed prevalence in the EU and Norway, 2004 – 2005 (*clean dataset*)

Member States	number of holdings [1]	SE				STM			
	N	N	%	95 CI lower limit	95 CI upper limit	N	%	95 CI lower limit	95 CI upper limit
AT	334	32	9.6	*7.4*	*12.2*	4	1.2	*0.5*	*2.5*
BE	130	34	26.2	*20.4*	*32.7*	1	0.8	*0.1*	*3.3*
CY	2	0	0.0	*0.0*	*67.1*	0	0.0	*0.0*	*67.1*
CZ	64	38	59.4	*54.9*	*62.1*	3	4.7	*2.9*	*6.6*
DE	522	120	23.0	*19.9*	*26.3*	10	1.9	*1.1*	*3.3*
DK	85	1	1.2	*0.1*	*5.1*	0	0.0	*0.0*	*3.1*
EE	11	1	9.1	*9.1*	*9.1*	0	0.0	*0.0*	*0.0*
EL	107	18	16.8	*11.7*	*23.5*	7	6.5	*3.5*	*11.6*
ES	481	232	48.2	*44.9*	*51.5*	26	5.4	*4.1*	*7.1*
FI	249	0	0.0	*0.0*	*0.9*	1	0.4	*0.0*	*1.6*
FR	511	20	3.9	*2.7*	*5.6*	22	4.3	*3.0*	*6.1*
HU	267	86	32.2	*28.6*	*35.9*	7	2.6	*1.6*	*4.2*
IE	146	0	0.0	*0.0*	*0.7*	0	0.0	*0.0*	*0.7*
IT	295	11	3.7	*2.3*	*6.1*	13	4.4	*2.8*	*6.9*
LT	8	4	50.0	*24.7*	*69.4*	0	0.0	*0.0*	*21.2*
LU	9	0	0.0	*0.0*	*0.0*	0	0.0	*0.0*	*0.0*
LV	6	0	0.0	*0.0*	*29.1*	0	0.0	*0.0*	*29.1*
NL	392	24	6.1	*4.4*	*8.5*	7	1.8	*0.9*	*3.3*
PL	290	159	54.8	*49.7*	*59.7*	7	2.4	*1.3*	*4.5*
PT	44	21	47.7	*34.9*	*60.4*	2	4.5	*1.3*	*13.7*
SWE	97	0	0.0	*0.0*	*2.8*	0	0.0	*0.0*	*2.8*
SI	98	9	9.2	*6.4*	*12.7*	0	0.0	*0.0*	*1.4*
UK	413	26	6.3	*4.6*	*8.5*	7	1.7	*0.9*	*3.0*
EU [2]	4,561	836	18.3			117	2.6		
EU weighted prevalence			**18.2**	***17.3***	***19.1***		**2.6**	***2.2***	***3.1***
NO	236	0	0.0	*0.0*	*1.2*	0	0.0	*0.00*	*1.2*

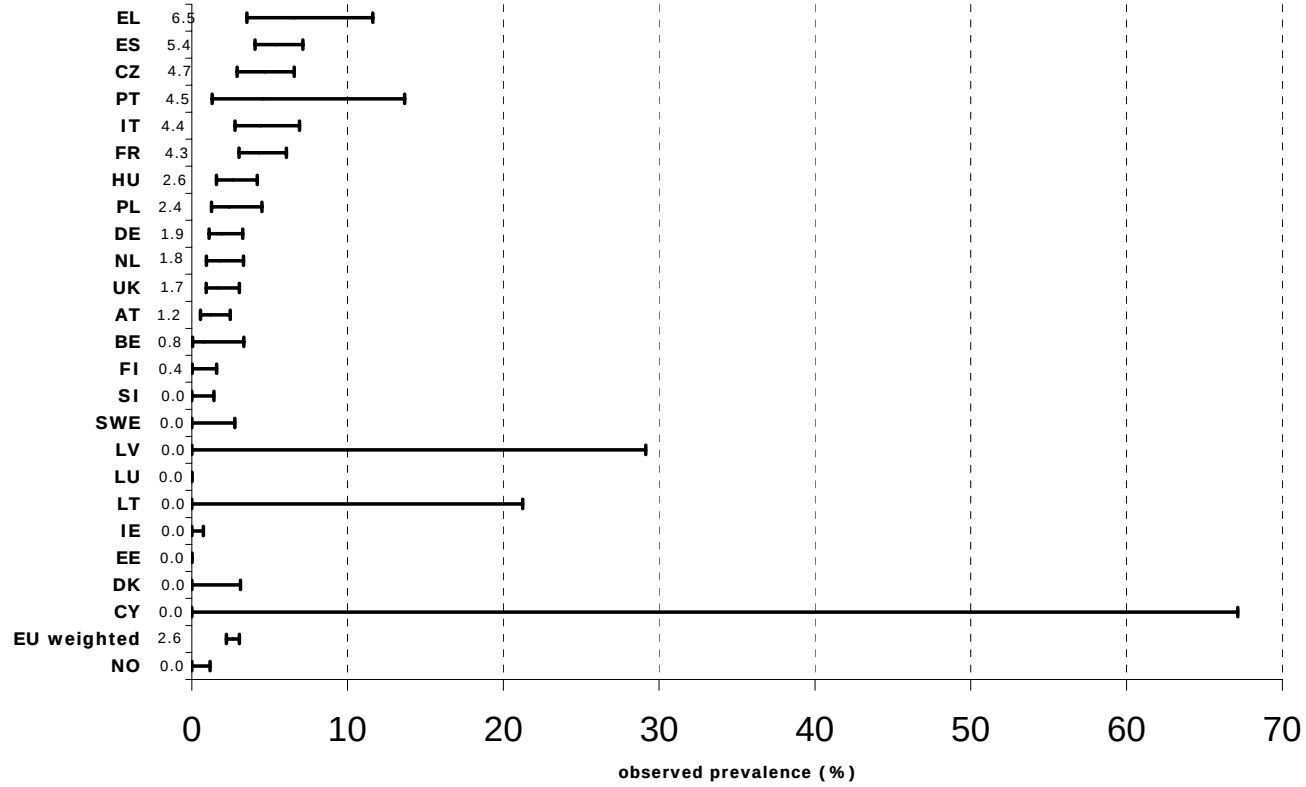
[1]: validated on the contents-level by EFSA

[2]: These EU figures do not include data for MT and SK

Annex VII. *Salmonella* Enteritidis holding observed prevalence 95% confidence intervals, for EU Member States, for the EU and for Norway, 2004 – 2005 (*clean dataset*)



Annex VIII. *Salmonella* Typhimurium holding observed prevalence 95% confidence intervals, for EU Member States, for the EU and for Norway, 2004 – 2005 (*clean dataset*)



Annex IX. *Salmonella* Enteritidis and *Salmonella* Typhimurium holding observed prevalence in the EU and Norway, 2004 – 2005 (*full dataset*)

Member States	Structure-level validated sample (COM)	SE				STM			
	N	N	%	95 CI lower limit	95 CI upper limit	N	%	95 CI lower limit	95 CI upper limit
AT	349	32	9.2	*7.2*	*11.7*	5	1.4	*0.7*	*2.7*
BE	150	41	27.3	*22.0*	*33.2*	1	0.7	*0.0*	*2.8*
CY	25	2	8.0	*3.7*	*12.3*	0	0.0	*0.0*	*2.9*
CZ	70	42	60.0	*60.0*	*60.0*	4	5.7	*5.7*	*5.7*
DE	564	132	23.4	*20.5*	*26.6*	11	2.0	*1.2*	*3.2*
DK	161	1	0.6	*0.0*	*2.1*	0	0.0	*0.0*	*1.1*
EE	11	1	9.1	*9.1*	*9.1*	0	0.0	*0.0*	*0.0*
EL	163	41	25.2	*20.5*	*30.3*	10	6.1	*3.9*	*9.4*
ES	507	246	48.5	*45.3*	*51.7*	26	5.1	*3.9*	*6.7*
FI	268	0	0.0	*0.0*	*0.8*	1	0.4	*0.0*	*1.4*
FR	524	20	3.8	*2.6*	*5.5*	22	4.2	*3.0*	*5.9*
HU	277	89	32.1	*28.7*	*35.6*	7	2.5	*1.5*	*4.0*
IE	156	0	0.0	*0.0*	*0.5*	0	0.0	*0.0*	*0.5*
IT	381	16	4.2	*2.8*	*6.2*	14	3.7	*2.4*	*5.6*
LT	11	4	36.4	*19.2*	*51.5*	0	0.0	*0.0*	*11.7*
LU	9	0	0.0	*0.0*	*0.0*	0	0.0	*0.0*	*0.0*
LV	6	0	0.0	*0.0*	*29.1*	0	0.0	*0.0*	*29.1*
NL	471	32	6.8	*5.1*	*8.9*	7	1.5	*0.8*	*2.7*
PL	362	198	54.7	*50.3*	*58.9*	9	2.5	*1.4*	*4.2*
PT	86	35	40.7	*32.8*	*48.7*	2	2.3	*0.6*	*6.4*
SWE	171	0	0.0	*0.0*	*1.3*	0	0.0	*0.0*	*1.3*
SI	108	12	11.1	*8.5*	*14.1*	0	0.0	*0.0*	*1.1*
SK	33	0	0.0	*0.0*	*2.5*	0	0.0	*0.0*	*2.5*
UK	454	28	6.2	*4.6*	*8.2*	8	1.8	*1.0*	*3.0*
EU[3]	5,317	972	18.3			127	2.4		
EU weighted prevalence			**18.4**	***17.6***	***19.2***		**2.5**	***2.1***	***2.8***
NO	314	0	0.0	*0*	*0.01*	0	0.0	*0*	*0.01*

[1]: Based on:
a)Technical specifications 'Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU'
Working document Sanco/34/2004 rev3, presented at the meeting of the Standing Committee on the Food Chain and Animal Health 15 July 2004
b) Upgraded data from Member States' final reports

[2]: Validated sampled proportion = actually sampled and validated by EFSA / Total * 100
In the following countries a small proportion of holdings were of size less than 1000 laying hens: CZ, IE, LU, SI

[3]: These EU figures do not include data for MT and SK

Annex X. *Salmonella* within-flock observed positive samples in the EU and Norway, 2004 – 2005 (*clean dataset*)

Table 9. *Salmonella* spp. within-flock observed positive samples in the EU and Norway, 2004 – 2005 (*clean dataset*)

Member States		*Salmonella* spp. within holding observed proportion of positive samples								
		Number of positive samples								
		Total	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
AT	Freq.	334	282	18	4	5	4	3	4	14
	Percent	*100.0*	*84.4*	*5.4*	*1.2*	*1.5*	*1.2*	*0.9*	*1.2*	*4.2*
BE	Freq.	130	84	11	5	2	7	7	6	8
	Percent	*100.0*	*64.6*	*8.5*	*3.8*	*1.5*	*5.4*	*5.4*	*4.6*	*6.2*
CY	Freq.	2	1				1			
	Percent	*100.0*	*50.0*				*33.3*			
CZ	Freq.	64	22	7	3	14	4	6	4	4
	Percent	*100.0*	*34.4*	*10.9*	*4.7*	*21.9*	*6.3*	*9.4*	*6.3*	*6.3*
DE	Freq.	522	372	31	29	20	19	18	19	14
	Percent	*100.0*	*71.3*	*5.9*	*5.6*	*3.8*	*3.6*	*3.4*	*3.6*	*2.7*
DK	Freq.	85	83			1	1			
	Percent	*100.0*	*97.6*			*1.2*	*1.2*			
EE	Freq.	11	9	1	1					
	Percent	*100.0*	*81.8*	*9.1*	*9.1*					
EL	Freq.	107	67	9	15	9	2	2	1	2
	Percent	*100.0*	*62.6*	*8.4*	*14.0*	*8.4*	*1.9*	*1.9*	*0.9*	*1.9*
ES	Freq.	481	129	46	54	29	27	41	47	108
	Percent	*100.0*	*26.8*	*9.6*	*11.2*	*6.0*	*5.6*	*8.5*	*9.8*	*22.5*
FI	Freq.	249	248	1						
	Percent	*100.0*	*99.6*	*0.4*						
FR	Freq.	511	423	28	19	9	10	6	7	9
	Percent	*100.0*	*82.8*	*5.5*	*3.7*	*1.8*	*2.0*	*1.2*	*1.4*	*1.8*
HU	Freq.	267	150	30	22	16	9	15	20	5
	Percent	*100.0*	*56.2*	*11.2*	*8.2*	*6.0*	*3.4*	*5.6*	*7.5*	*1.9*
IE	Freq.	146	144	2						
	Percent	*100.0*	*98.6*	*1.4*						
IT	Freq.	295	206	29	15	14	5	5	8	13
	Percent	*100.0*	*69.8*	*9.8*	*5.1*	*4.7*	*1.7*	*1.7*	*2.7*	*4.4*
LT	Freq.	8	4	2	1			1		
	Percent	*100.0*	*50.0*	*25.0*	*12.5*			*12.5*		
LU	Freq.	9	9							
	Percent	*100.0*	*100.0*							
LV	Freq.	6	5	1						
	Percent	*100.0*	*83.3*	*16.7*						
NL	Freq.	392	330	21	10	7	5	6	6	7
	Percent	*100.0*	*84.2*	*5.4*	*2.6*	*1.8*	*1.3*	*1.5*	*1.5*	*1.8*
PL	Freq.	290	66	30	28	19	24	41	28	54
	Percent	*100.0*	*22.8*	*10.3*	*9.7*	*6.6*	*8.3*	*14.1*	*9.7*	*18.6*
PT	Freq.	44	9	2	9	4	5	4	4	7
	Percent	*100.0*	*20.5*	*4.5*	*20.5*	*9.1*	*11.4*	*9.1*	*9.1*	*15.9*
SWE	Freq.	97	97							
	Percent	*100.0*	*100.0*							
SI	Freq.	98	79	5	3	3	1	3	2	2
	Percent	*100.0*	*80.6*	*5.1*	*3.1*	*3.1*	*1.0*	*3.1*	*2.0*	*2.0*
UK	Freq.	413	364	15	6	7	7	2	6	6
	Percent	*100.0*	*88.1*	*3.6*	*1.5*	*1.7*	*1.7*	*0.5*	*1.5*	*1.5*
EU[3]	Freq.	4,561	3,183	289	224	159	131	160	162	253
	Percent	*100*	*69.8*	*6.3*	*4.9*	*3.5*	*2.9*	*3.5*	*3.6*	*5.5*
NO	Freq.	236	236							
	Percent	*100*	*100.0*							

[1]: Based on:
a) Technical specifications 'Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU', Working document Sanco/34/2004 rev3, presented at the meeting of the Standing Committee on the Food Chain and Animal Health, 15 July 2004
b) Upgraded data from Member States' final reports

[2]: Sampled proportion = actually sampled / Total * 100
In the following countries a small proportion of holdings were of size less than 1000 laying hens: CZ, IE (22/165), LU, SI

[3]: These EU-25 figures do not include data for MT and SK

Table 10. *Salmonella* Enteritidis / Typhimurium within-flock observed positive samples in the EU and Norway, 2004 – 2005 (*clean dataset*)

Member States		*Salmonella* Enteritidis / Typhimurium within holding observed proportion of positive samples								
		Number of positive samples								
		Total	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
AT	Freq.	334	298	9	4	5	3	2	3	10
	Percent	*100.0*	*89.2*	*2.7*	*1.2*	*1.5*	*0.9*	*0.6*	*0.9*	*3.0*
BE	Freq.	130	96	8	3	3	7	6	7	
	Percent	*100.0*	*73.8*	*6.2*	*2.3*	*2.3*	*5.4*	*4.6*	*5.4*	*0.0*
CY	Freq.	2	2							
	Percent	*100.0*	*100.0*							
CZ	Freq.	64	24	8	4	11	4	6	4	3
	Percent	*100.0*	*37.5*	*12.5*	*6.3*	*17.2*	*6.3*	*9.4*	*6.3*	*4.7*
DE	Freq.	522	395	31	30	27	14	12	7	6
	Percent	*100.0*	*75.7*	*5.9*	*5.7*	*5.2*	*2.7*	*2.3*	*1.3*	*1.1*
DK	Freq.	85	84			1				
	Percent	*100.0*	*98.8*			*1.2*				
EE	Freq.	11	10		1					
	Percent	*100.0*	*90.9*		*9.1*					
EL	Freq.	107	83	12	4	4	1	1	2	
	Percent	*100.0*	*77.6*	*11.2*	*3.7*	*3.7*	*0.9*	*0.9*	*1.9*	
ES	Freq.	481	233	59	55	35	25	28	21	25
	Percent	*100.0*	*48.4*	*12.3*	*11.4*	*7.3*	*5.2*	*5.8*	*4.4*	*5.2*
FI	Freq.	249	248	1						
	Percent	*100.0*	*99.6*	*0.4*						
FR	Freq.	511	470	15	9	3	5	2	2	5
	Percent	*100.0*	*92.0*	*2.9*	*1.8*	*0.6*	*1.0*	*0.4*	*0.4*	*1.0*
HU	Freq.	267	177	23	19	8	11	11	15	3
	Percent	*100.0*	*66.3*	*8.6*	*7.1*	*3.0*	*4.1*	*4.1*	*5.6*	*1.1*
IE	Freq.	146	146							
	Percent	*100.0*	*100.0*							
IT	Freq.	295	271	13	4	3	1		1	2
	Percent	*100.0*	*91.9*	*4.4*	*1.4*	*1.0*	*0.3*		*0.3*	*0.7*
LT	Freq.	8	4	2	1		1			
	Percent	*100.0*	*50.0*	*25.0*	*12.5*		*12.5*			
LU	Freq.	9	9							
	Percent	*100.0*	*100.0*							
LV	Freq.	6	6							
	Percent	*100.0*	*100.0*							
NL	Freq.	392	361	8	6	2	5	3	4	3
	Percent	*100.0*	*92.1*	*2.0*	*1.5*	*0.5*	*1.3*	*0.8*	*1.0*	*0.8*
PL	Freq.	290	128	33	27	14	22	34	14	18
	Percent	*100.0*	*44.1*	*11.4*	*9.3*	*4.8*	*7.6*	*11.7*	*4.8*	*6.2*
PT	Freq.	44	23	6	6	2	1	2	3	1
	Percent	*100.0*	*52.3*	*13.6*	*13.6*	*4.5*	*2.3*	*4.5*	*6.8*	*2.3*
SWE	Freq.	97	97							
	Percent	*100.0*	*100.0*							
SI	Freq.	98	89		1	2		2	2	2
	Percent	*100.0*	*90.8*		*1.0*	*2.0*		*2.0*	*2.0*	*2.0*
UK	Freq.	413	380	7	5	4	4	2	7	4
	Percent	*100.0*	*92.0*	*1.7*	*1.2*	*1.0*	*1.0*	*0.5*	*1.7*	*1.0*
EU[3]	Freq.	4,561	3,634	235	179	124	104	111	92	82
	Percent	*100.0*	*79.7*	*5.2*	*3.9*	*2.7*	*2.3*	*2.4*	*2.0*	*1.8*
NO	Freq.	236	236							
	Percent	*100.0*	*100.0*							

[1]: Based on:
a) Technical specifications 'Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU', Working document Sanco/34/2004 rev3, presented at the meeting of the Standing Committee on the Food Chain and Animal Health, 15 July 2004
b) Upgraded data from Member States' final reports

[2]: Sampled proportion = actually sampled / Total * 100
In the following countries a small proportion of holdings were of size less than 1000 laying hens: CZ, IE (22/165), LU, SI

[3]: These EU-25 figures do not include data for MT and SK

Annex XI. Frequency of isolated *Salmonella* serovars, in the EU Member States and Norway, 2004 – 2005

Austria	Serovar name	Number of isolates	Percent
	S. Enteritidis	158	70.85
	S. Infantis	15	6.73
	S. Typhimurium	12	5.38
	S. Montevideo	11	4.93
	S. Tennessee	9	4.04
	S. Mbandaka	7	3.14
	S. Braenderup	5	2.24
	S. Senftenberg	2	0.90
	S. IIIb 61 : k : 1,5,7	2	0.90
	S. Bredeney	1	0.45
	S. Agona	1	0.45
	Others	0	0.00
	Total	223	100.00

Belgium	Serovar name	Number of isolates	Percent
	S. Enteritidis	122	66.30
	S. Mbandaka	10	5.43
	S. Braenderup	10	5.43
	S. Agona	6	3.26
	S. Rissen	4	2.17
	S. Livingstone	4	2.17
	S.03, 19 : G,S,T	4	2.17
	S. Montevideo	3	1.63
	S. Indiana	2	1.09
	S. 03, 19 : G,S,T	2	1.09
	no typification	2	1.09
	Others	15	8.15
	Total	184	100.00

Cyprus	Serovar name	Number of isolates	Percent
	S. Newport	4	100.00
	Others	0	0.00
	Total	4	100.00

Czech Republic	Serovar name	Number of isolates	Percent
	S. Enteritidis	132	86.27
	S. Typhimurium	8	5.23
	S. Saint Paul	7	4.58
	S. Infantis	4	2.61
	S. Schwarzengrund	1	0.65
	S. Lille	1	0.65
	Others	0	0.00
	Total	153	100.00

Germany	Serovar name	Number of isolates	Percent
	S. Enteritidis	348	64.93
	S.Subspec. I Rauform	94	17.54
	S. Typhimurium	28	5.22
	S. Infantis	20	3.73
	S. Livingstone	10	1.87
	S. der Gruppe D1	6	1.12
	S. Rissen	5	0.93
	S. Mbandaka	5	0.93
	S. Hadar	4	0.75
	S. Tennessee	3	0.56
	S. der Gruppe E1	3	0.56
	Others	10	1.87
	Total	536	100.00

Denmark	Serovar name	Number of isolates	Percent
	S. Infantis	4	57.14
	S. Enteritidis	3	42.86
	Others	0	0.00
	Total	7	100.00

Estonia	Serovar name	Number of isolates	Percent
	S. Enteritidis	2	66.67
	Salmonella Isangi	1	33.33
	Others	0	0.00
	Total	3	100.00

Greece	Serovar name	Number of isolates	Percent
	S. Enteritidis	40	38.10
	other specify	22	20.95
	S. Typhimurium	14	13.33
	S. Livingstone	6	5.71
	S. Braenderup	6	5.71
	S. Infantis	5	4.76
	S. ISANGI	4	3.81
	S. Virchow	2	1.90
	S. PAPUANA	2	1.90
	S. Agona	2	1.90
	Others	2	1.90
	Total	105	100.00

Spain	Serovar name	Number of isolates	Percent
	S. Enteritidis	756	47.49
	S. Infantis	149	9.36
	S. Ohio	100	6.28
	S. Typhimurium	59	3.71
	S. Livingstone	58	3.64
	S. Hadar	47	2.95
	S. Mbandaka	36	2.26
	S. Virchow	31	1.95
	S. Montevideo	27	1.70
	S. Altona	21	1.32
	S. Bredeney	21	1.32
	Others	287	18.03
	Total	1592	100.00

Finland	Serovar name	Number of isolates	Percent
	S. Typhimurium	1	100.00
	Others	0	0.00
	Total	1	100.00

France	Serovar name	Number of isolates	Percent
	S. Enteritidis	125	23.72
	S. Typhimurium	107	20.30
	S. Infantis	46	8.73
	S. Tennessee	30	5.69
	S. Montevideo	28	5.31
	S. Braenderup	28	5.31
	S. Mbandaka	27	5.12
	S. Virchow	26	4.93
	S. Livingstone	25	4.74
	S. Anatum	15	2.85
	Others	70	13.28
	Total	527	100.00

Hungary	Serovar name	Number of isolates	Percent
	S. Enteritidis	340	73.59
	S. Infantis	19	4.11
	S. Bovismorbificans	16	3.46
	S. Agona	15	3.25
	S. Mbandaka	14	3.03
	S. Typhimurium	13	2.81
	S. Bredeney	10	2.16
	S. spp enterica O:9,12 H:l? :	5	1.08
	S. Newport	4	0.87
	S. Derby	4	0.87
	Others	22	4.76
	Total	462	100.00

Ireland	Serovar name	Number of isolates	Percent
	S. Reading	1	50.00
	S. Brandenburg	1	50.00
	Others	0	0.00
	Total	2	100.00

Italy	Serovar name	Number of isolates	Percent
	S. Hadar	35	11.71
	S. Enteritidis	30	10.03
	S. Typhimurium	24	8.03
	S. Bredeney	23	7.69
	S. Mbandaka	17	5.69
	S. Infantis	16	5.35
	S. Livingstone	12	4.01
	S. Thompson	10	3.34
	S. Braenderup	9	3.01
	S. Virchow	8	2.68
	S. Kentucky	8	2.68
	Others	107	35.79
	Total	299	100.00

Lithuania	Serovar name	Number of isolates	Percent
	S. Enteritidis	8	88.89
	Salmonella spp.	1	11.11
	Others	0	0.00
	Total	9	100.00

Latvia	Serovar name	Number of isolates	Percent
	other specify - S. Santemarie	1	100.00
	Others	0	0.00
	Total	1	100.00

The Netherlands	Serovar name	Number of isolates	Percent
	S. Enteritidis	93	47.21
	S. Typhimurium	13	6.60
	S. Senftenberg	11	5.58
	S. Virchow	10	5.08
	S. Agona	10	5.08
	S. Livingstone	10	5.08
	S. Duisburg	8	4.06
	S. Braenderup	7	3.55
	S. Paratyphi B var Java	5	2.54
	S. Montevideo	5	2.54
	Others	25	12.69
	Total	197	100.00

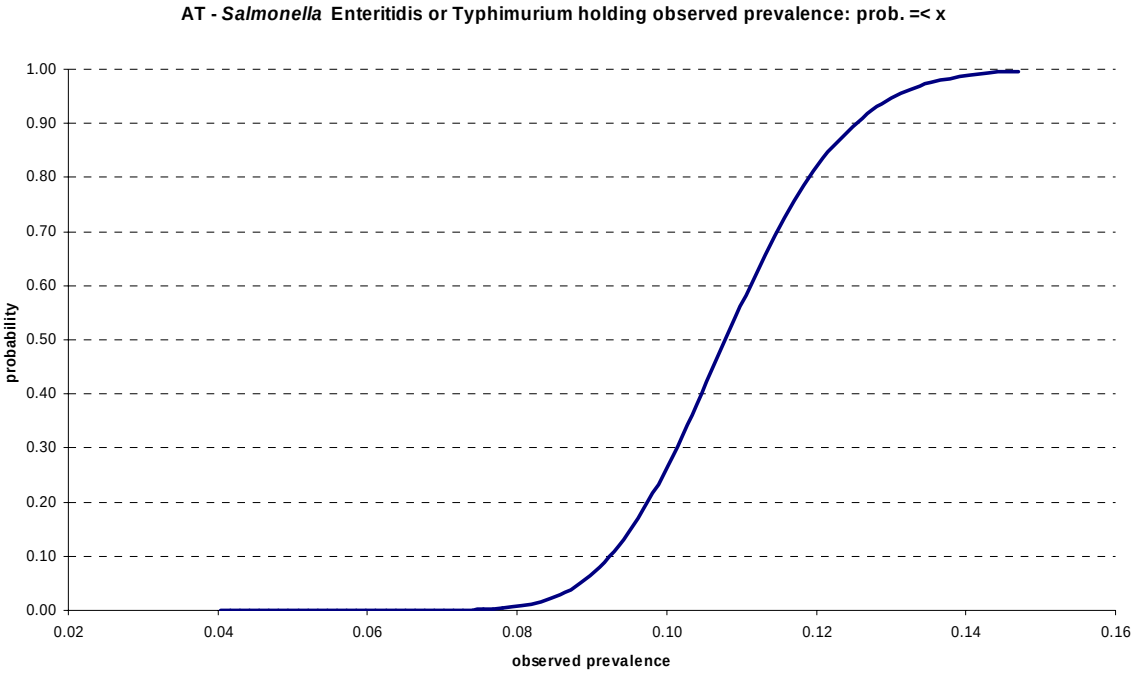
Poland	Serovar name	Number of isolates	Percent
	S. Enteritidis	585	59.03
	S. Infantis	185	18.67
	S. Mbandaka	65	6.56
	S. Virchow	59	5.95
	S. Hadar	21	2.12
	S. Livingstone	12	1.21
	S. Typhimurium	12	1.21
	S. Schwarzengrund	8	0.81
	S. Indiana	8	0.81
	S. Montevideo	7	0.71
	S. Anatum	7	0.71
	Others	22	2.22
	Total	991	100.00

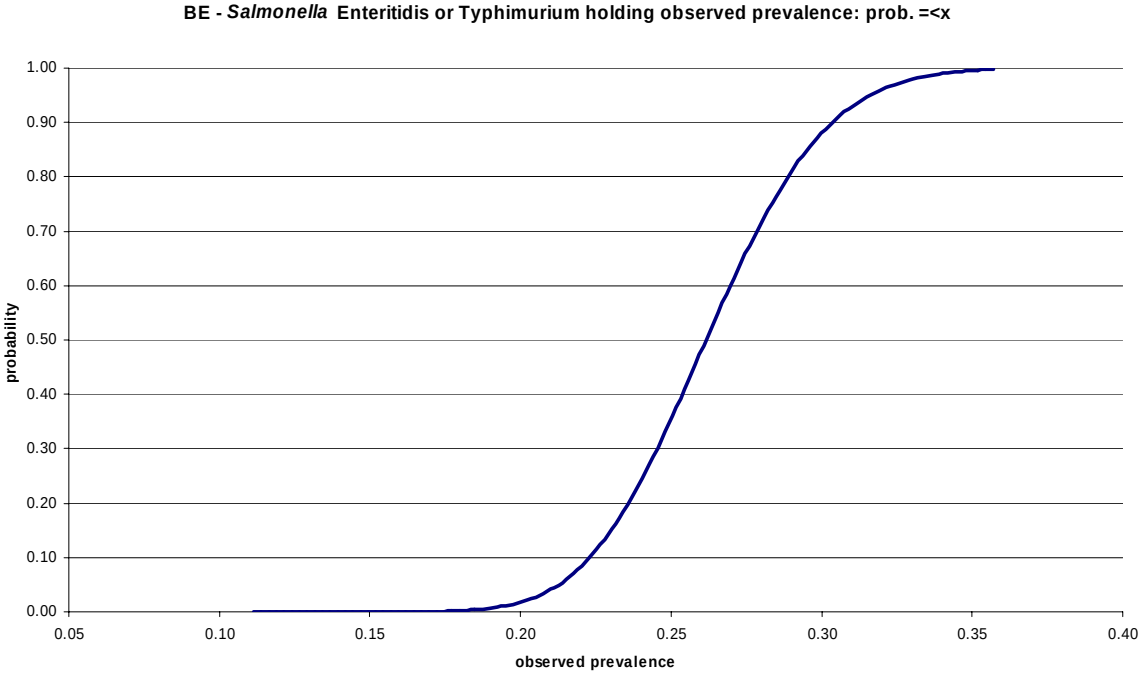
Portugal	Serovar name	Number of isolates	Percent
	S. Enteritidis	59	40.69
	S. Mbandaka	49	33.79
	S. Heidelberg	12	8.28
	S. Tennessee	6	4.14
	S. Agona	5	3.45
	S. Typhimurium	4	2.76
	other specify	3	2.07
	Salmonella Give	1	0.69
	S. Rugosa	1	0.69
	S. Rissen	1	0.69
	S. Livingstone	1	0.69
	S. Havana	1	0.69
	S. Hadar	1	0.69
	S. -:g,m:-	1	0.69
	Others	0	0.00
	Total	145	100.00

Slovenia	Serovar name	Number of isolates	Percent
	S. Enteritidis	44	67.69
	S. Menden	5	7.69
	S. Infantis	5	7.69
	S. Rissen	4	6.15
	S. Agona	3	4.62
	S. Stanleyville	2	3.08
	S. Mbandaka	1	1.54
	S. Heidelberg	1	1.54
	Others	0	0.00
	Total	65	100.00

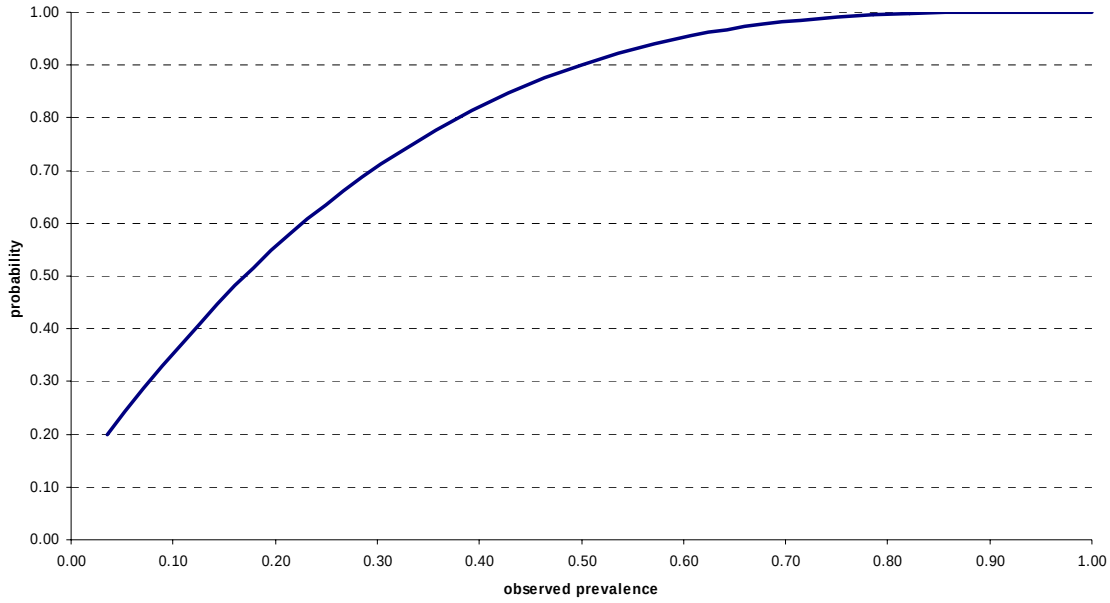
United Kingdom	Serovar name	Number of isolates	Percent
	S. Enteritidis	105	64.02
	S. Typhimurium	20	12.20
	S. Mbandaka	7	4.27
	S. Livingstone	6	3.66
	S. Senftenberg	4	2.44
	S. Thompson	3	1.83
	S. Agona	2	1.22
	S. Corvallis	2	1.22
	S. Cubana	2	1.22
	S. Kentucky	2	1.22
	Others	11	6.71
	Total	164	100.00

Annex XII. Probability graph: SE or STM holding observed prevalence in the EU Member States and Norway =< x, 2004 – 2005 (*clean dataset*)

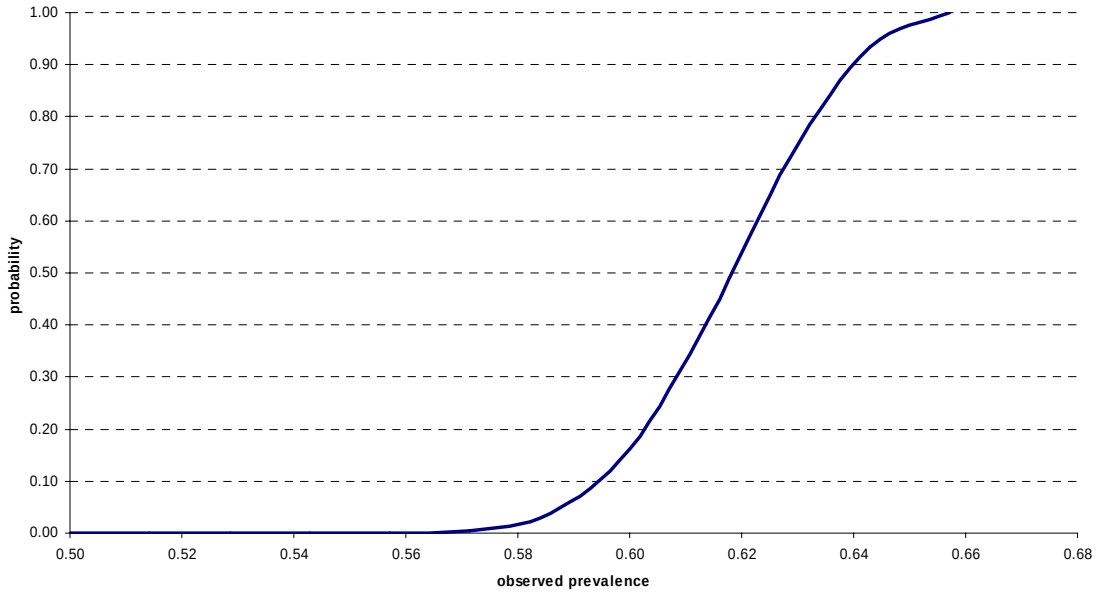




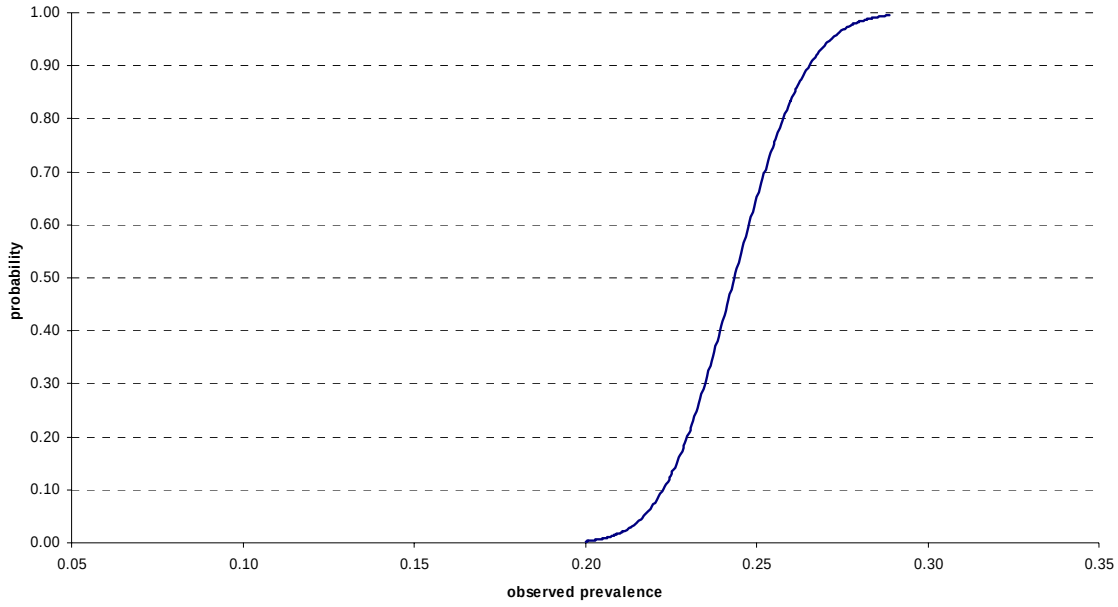
CY - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =<x



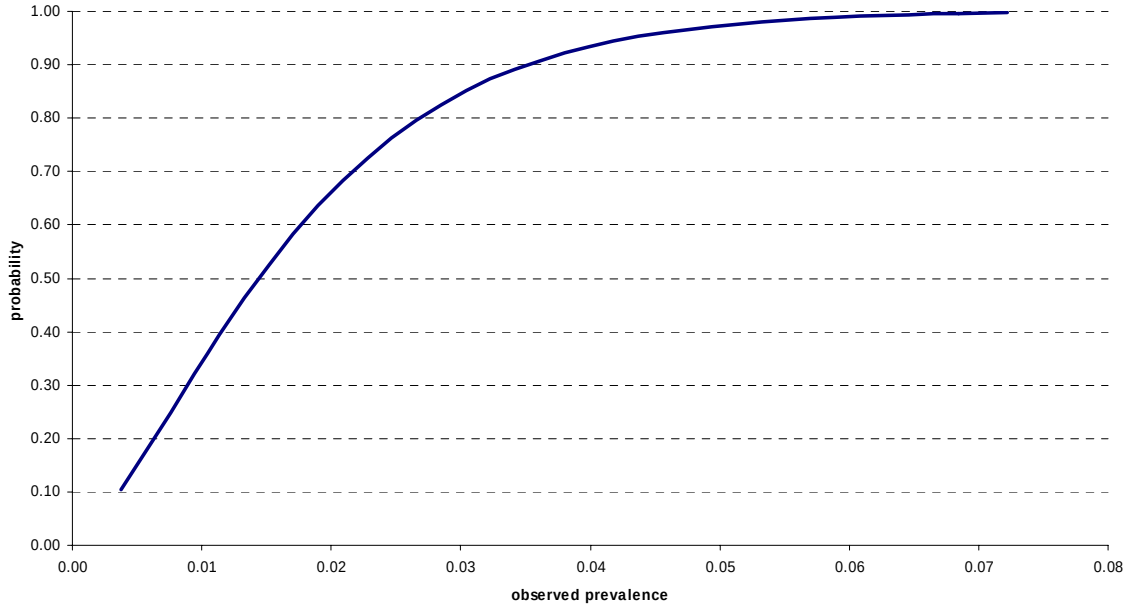
CZ - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =<x



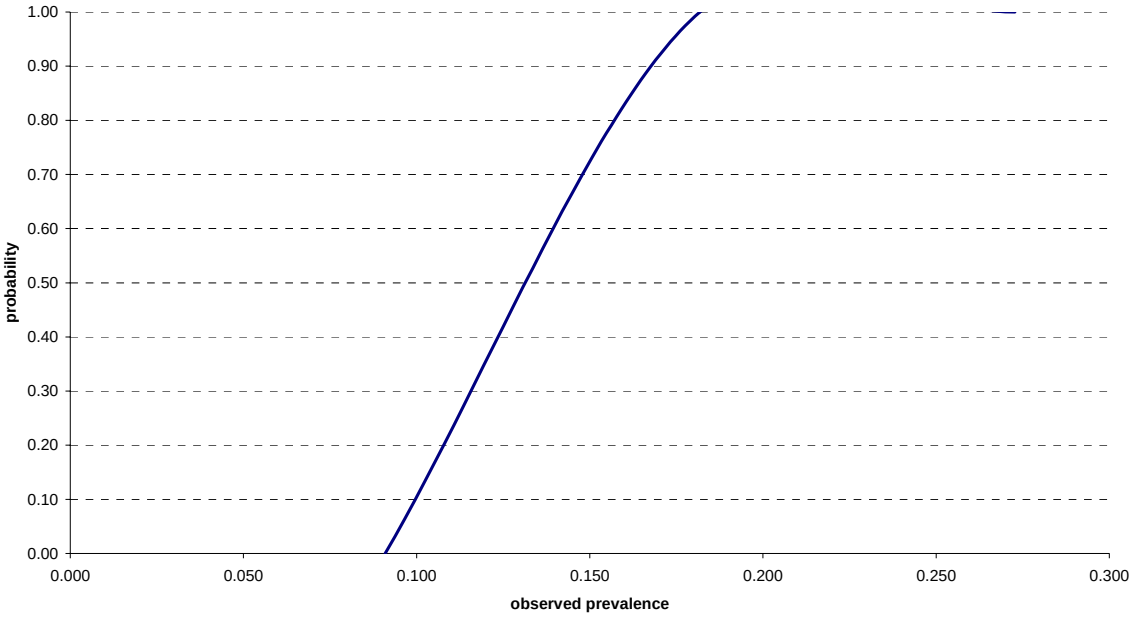
DE - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =< x



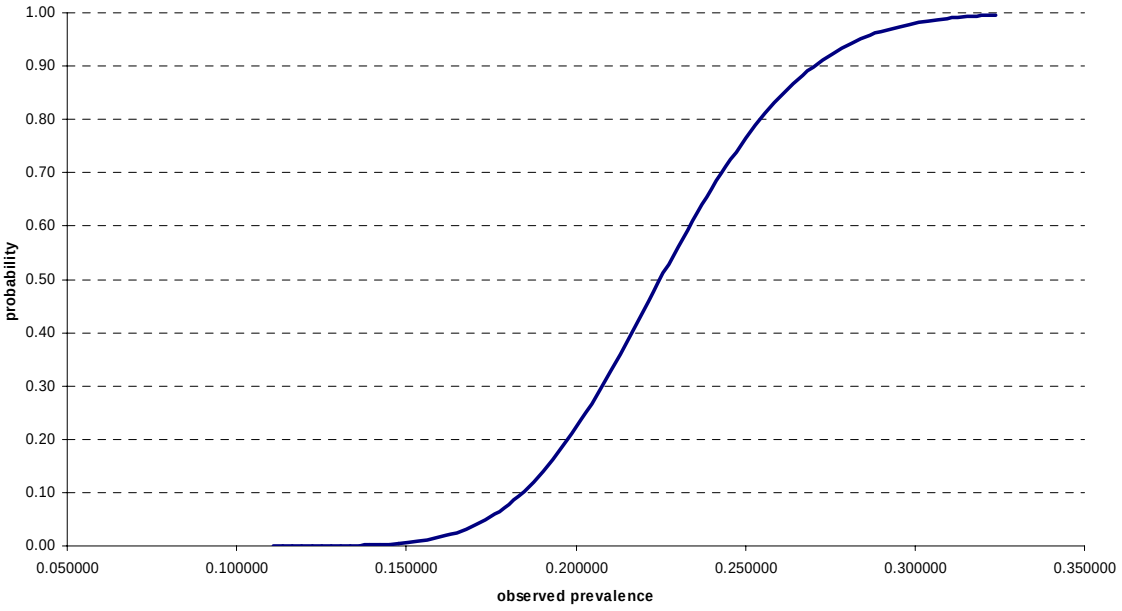
DK - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =<x



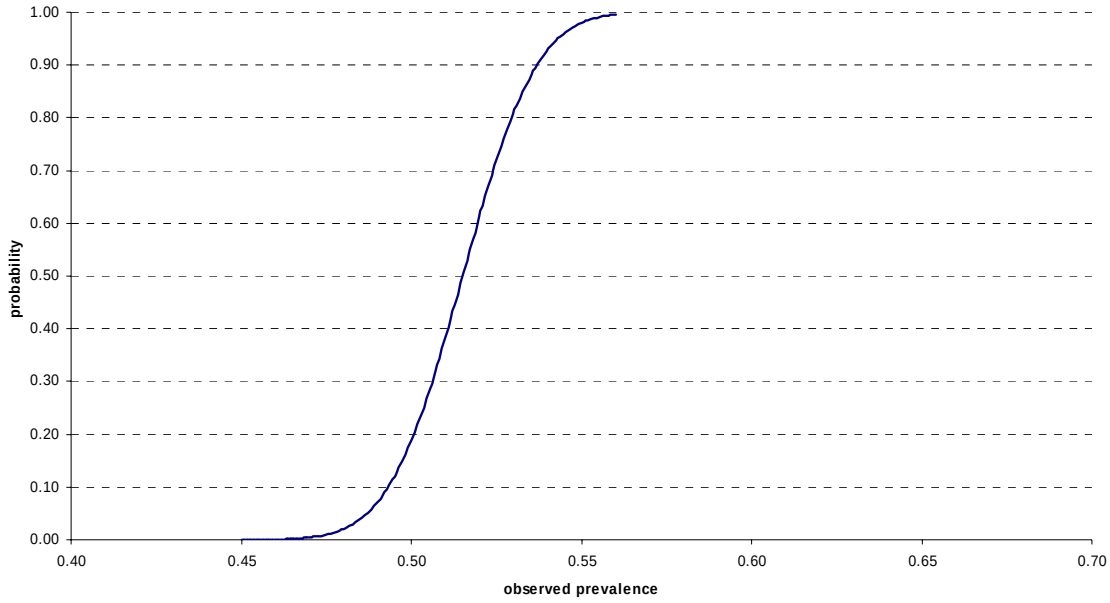
EE - *Salmonella* spp. holding observed prevalence: prob. =<x



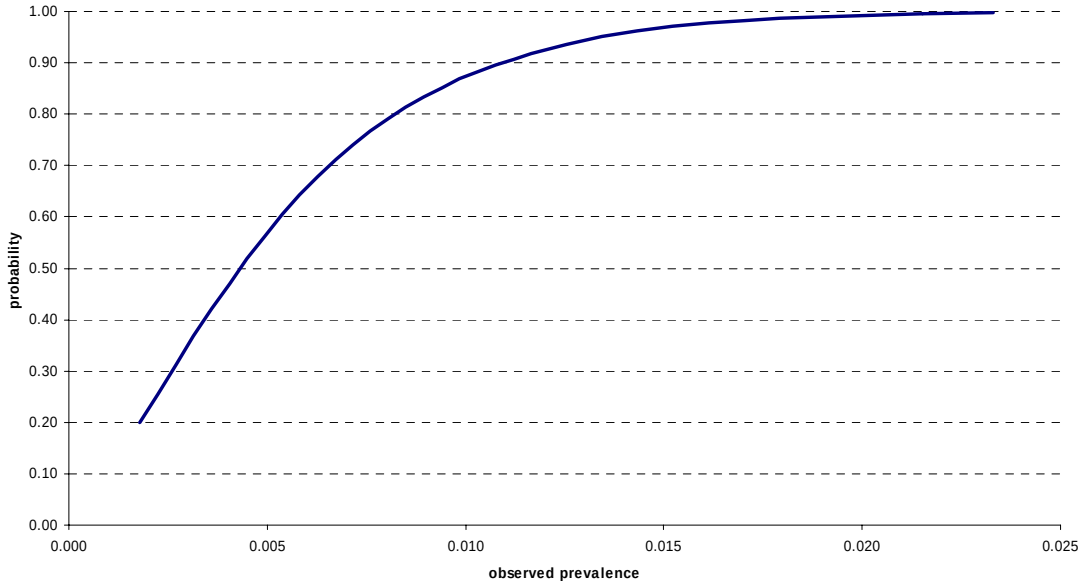
EL - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =<x



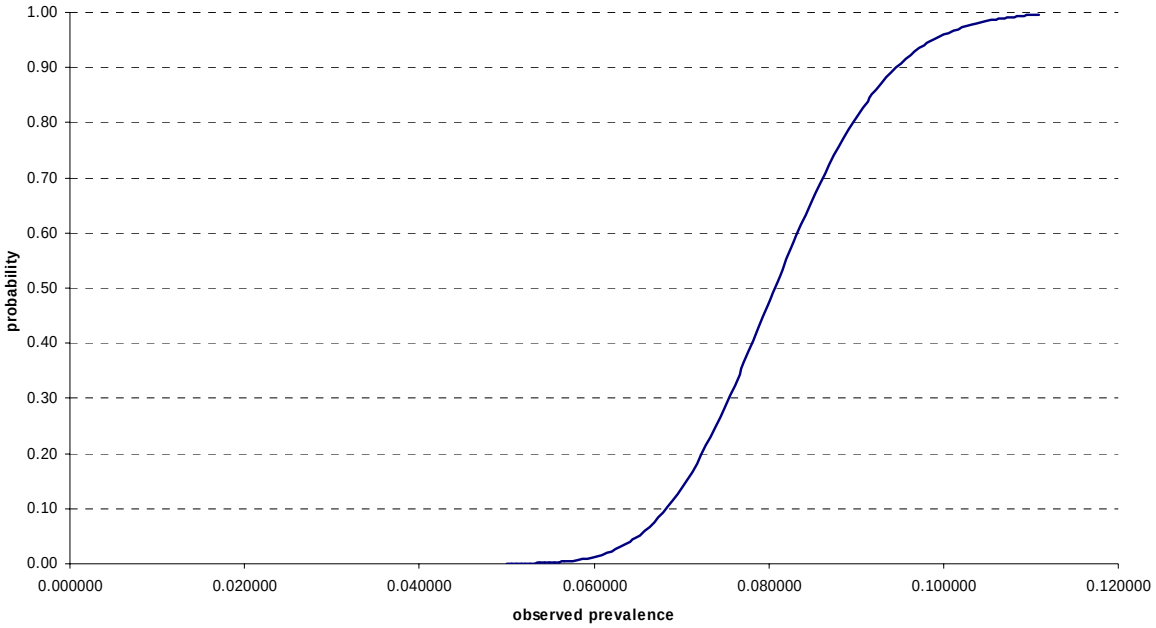
ES - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =< x



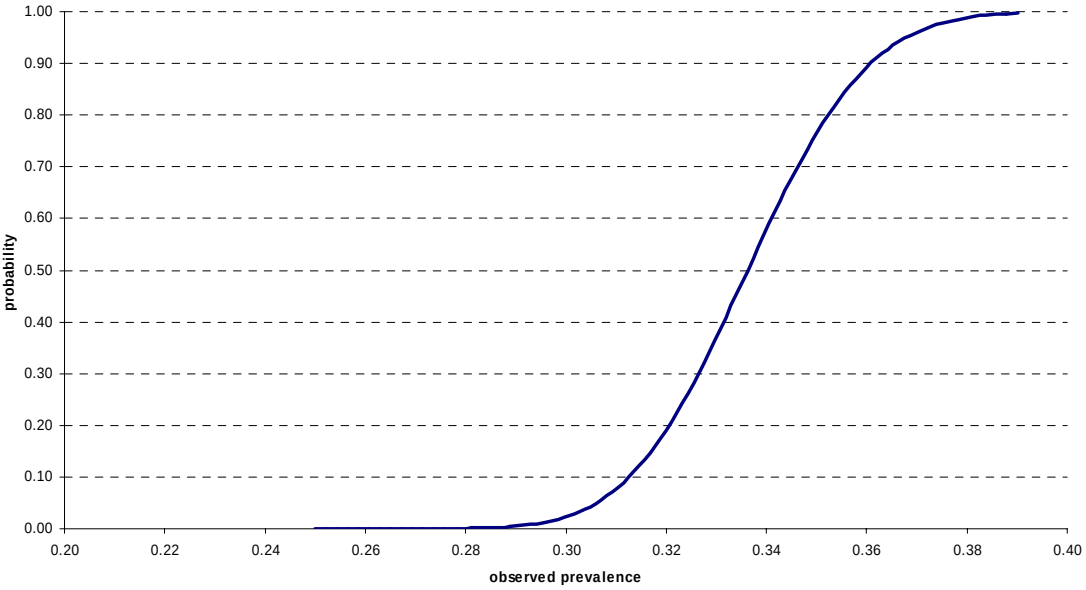
FI - Salm spp. or Salm Enteritidis-or-Typhimurium holding observed prevalence: prob. =<x

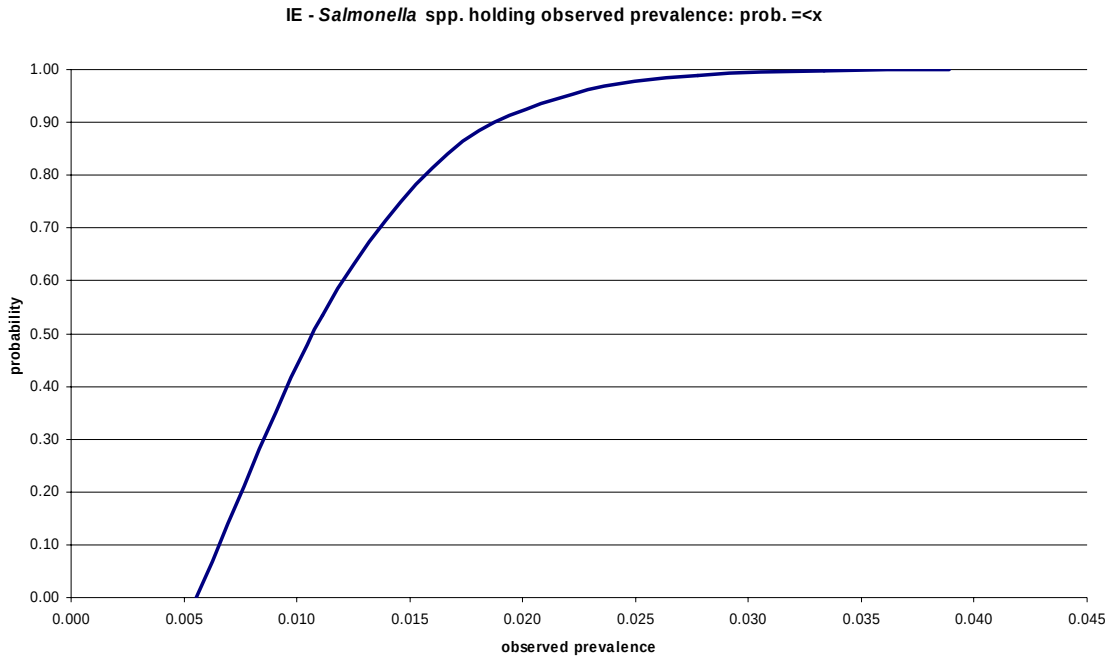


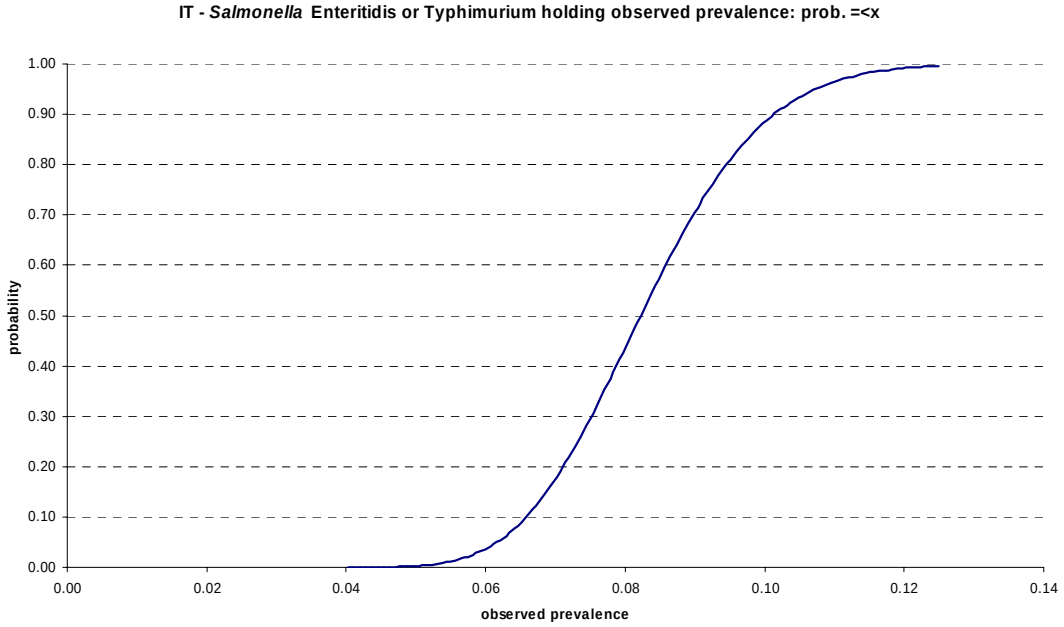
FR - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =< x



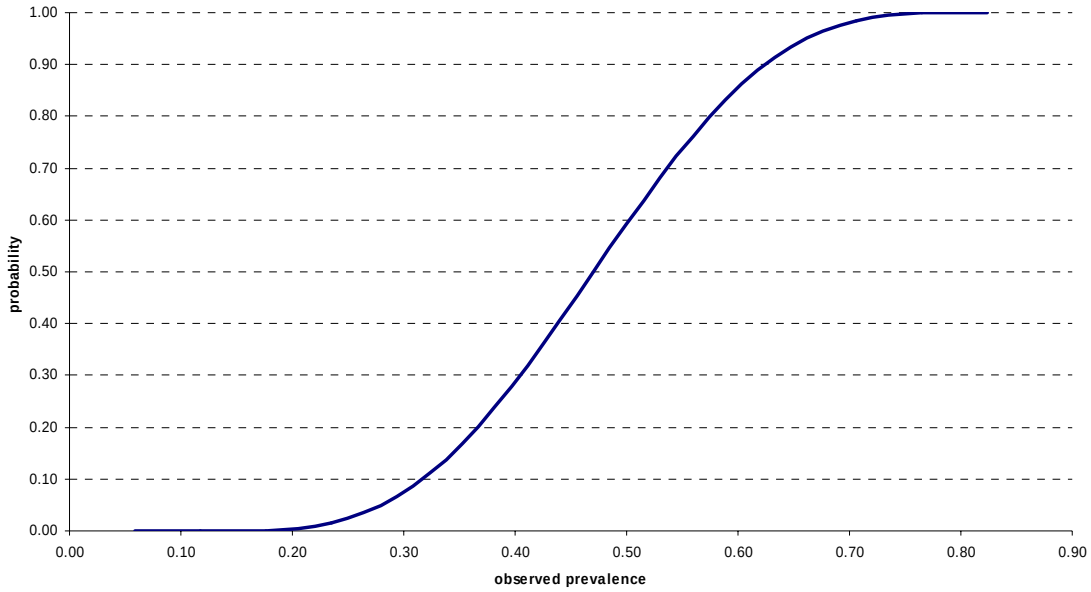
HU - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =<x



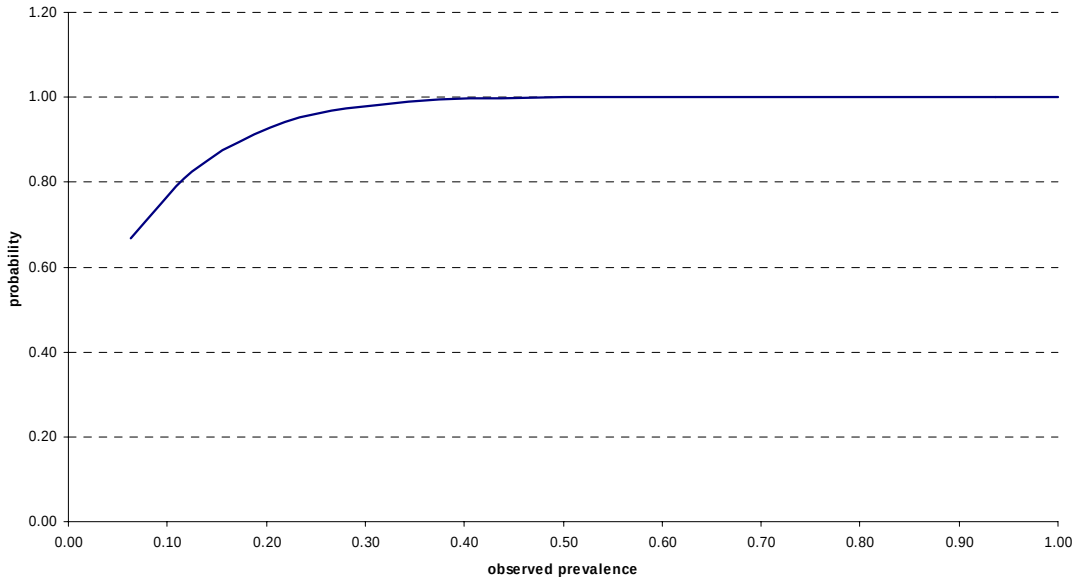

IE - Salmonella spp. holding observed prevalence: prob. =<x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.035
0.040
0.045
observed prevalence

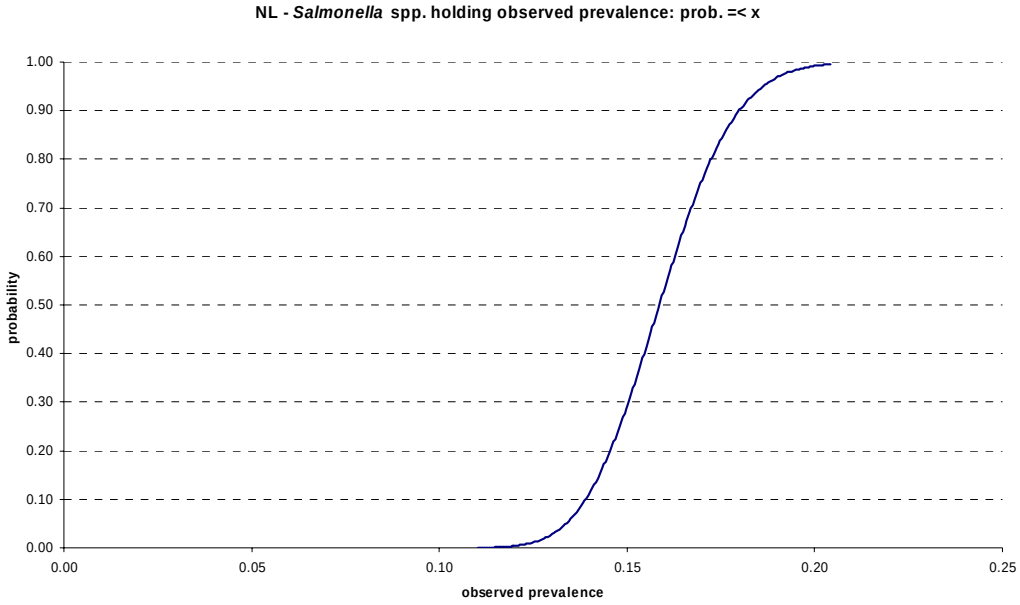

IT - Salmonella Enteritidis or Typhimurium holding observed prevalence: prob. =<x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
observed prevalence

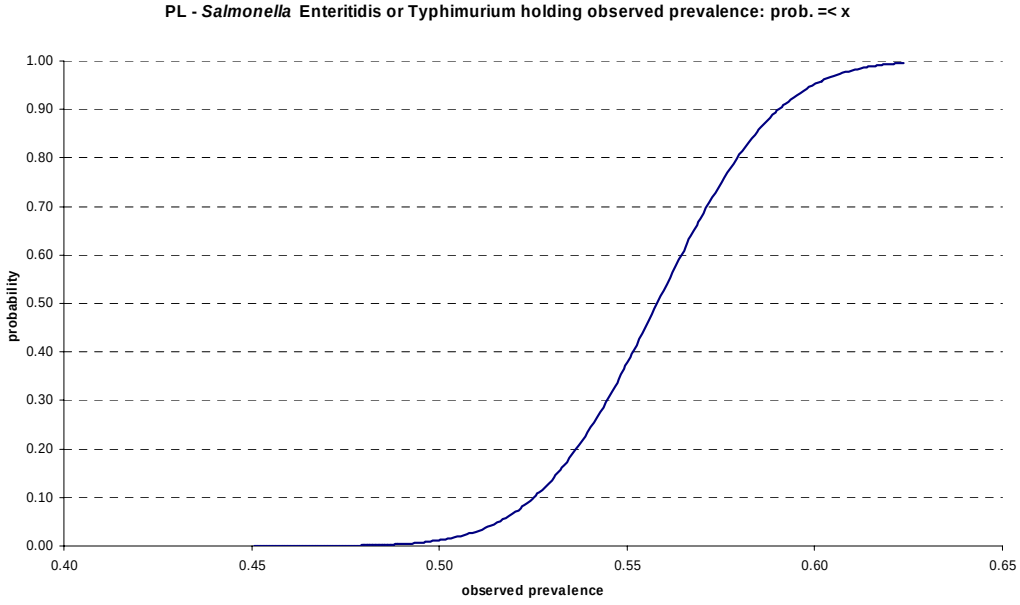
LT - *Salmonella* spp. or Enteritidis-or-Typhimurium holding observed prevalence: prob. =< x

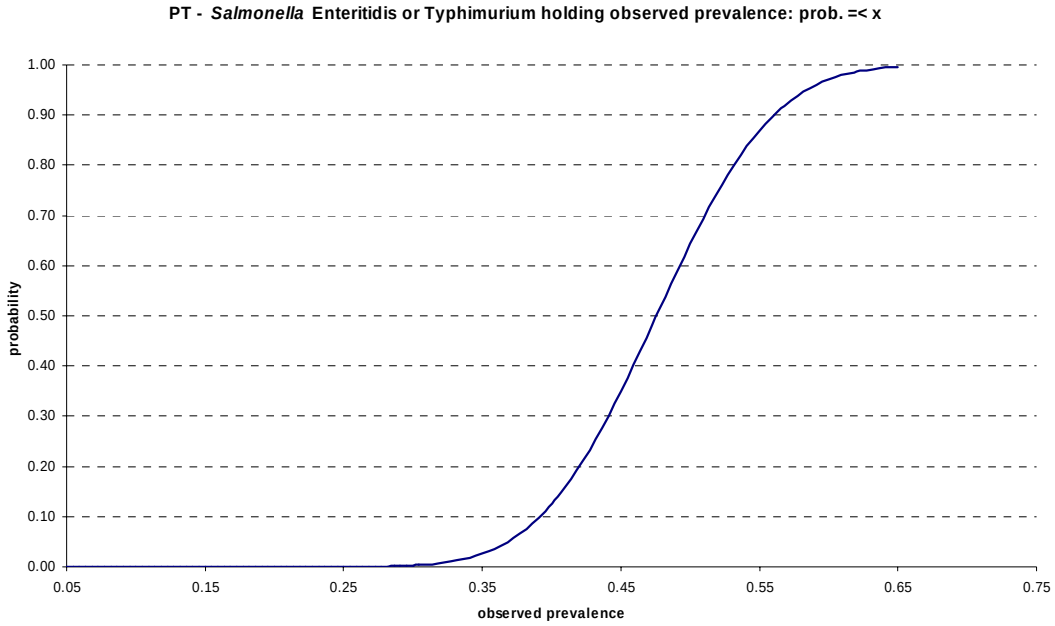


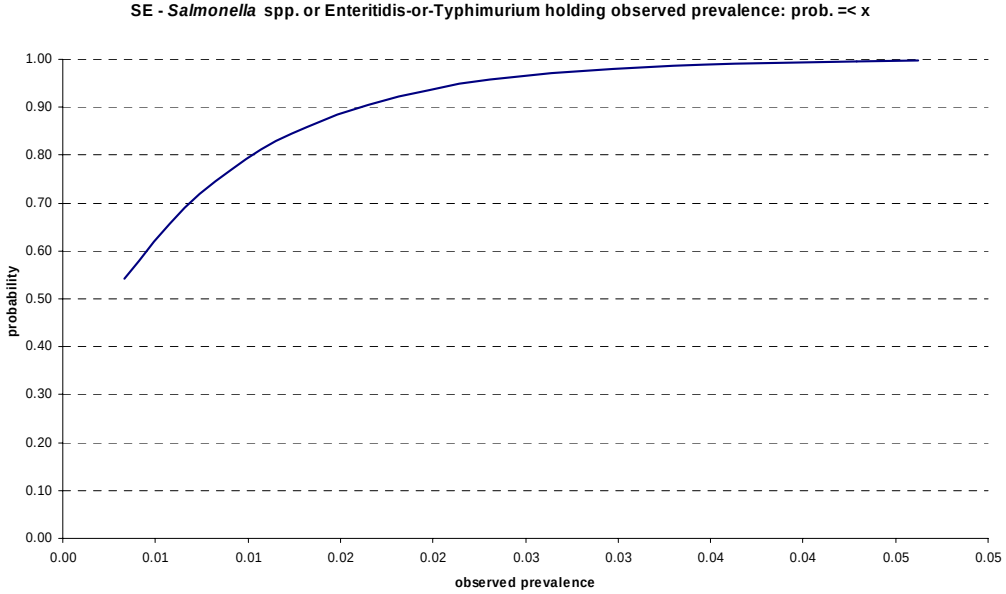
LV - *Salmonella* Enteritidis or Typhimurium holding observed prevalence: prob. =<x



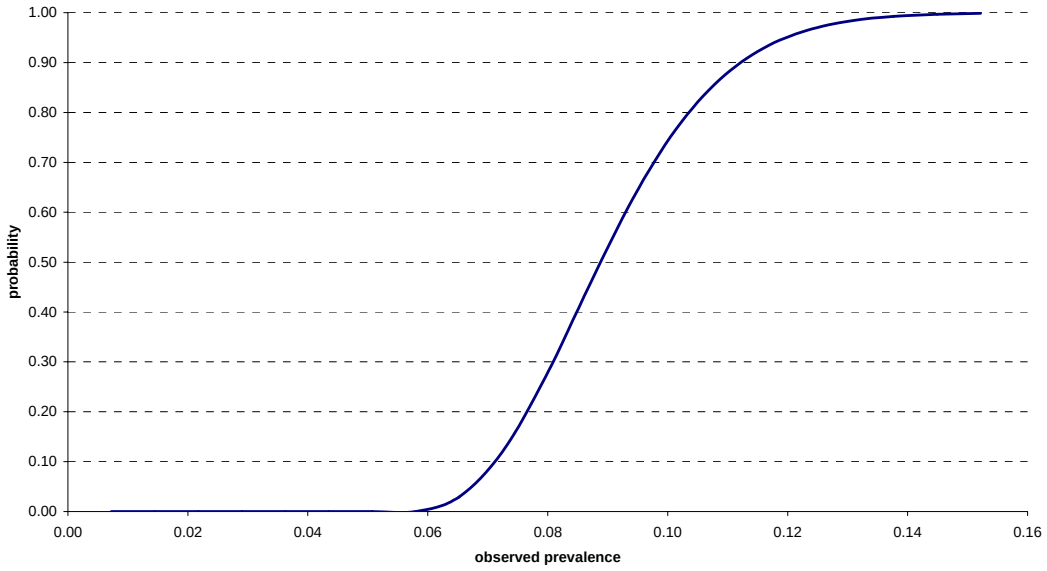

NL - Salmonella spp. holding observed prevalence: prob. =< x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.05
0.10
0.15
0.20
0.25
observed prevalence


PL - Salmonella Enteritidis or Typhimurium holding observed prevalence: prob. =< x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.40
0.45
0.50
0.55
0.60
0.65
observed prevalence

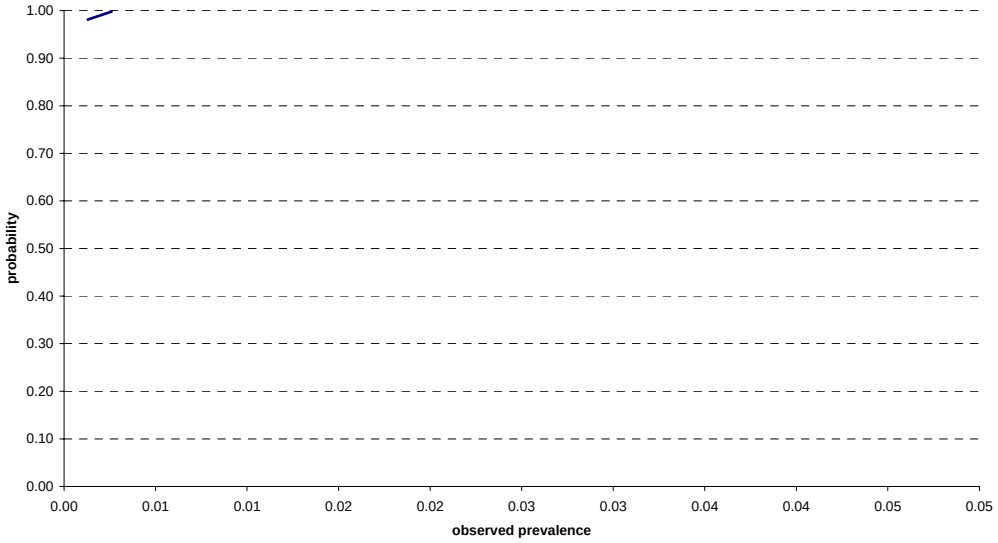

PT - Salmonella Enteritidis or Typhimurium holding observed prevalence: prob. =< x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.05
0.15
0.25
0.35
0.45
0.55
0.65
0.75
observed prevalence

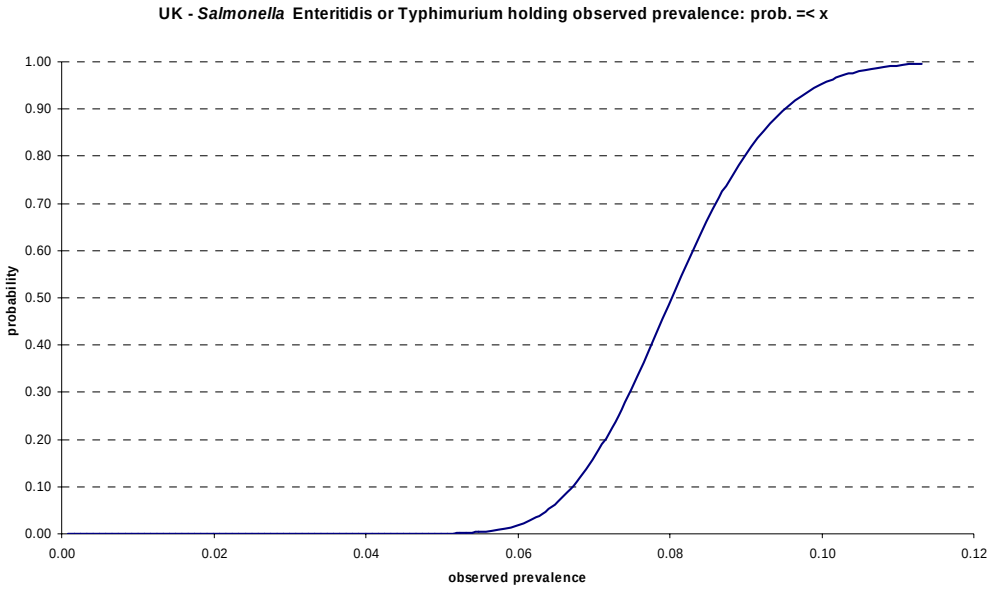

SE - Salmonella spp. or Enteritidis-or-Typhimurium holding observed prevalence: prob. =< x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.01
0.01
0.02
0.02
0.03
0.03
0.04
0.04
0.05
0.05
observed prevalence

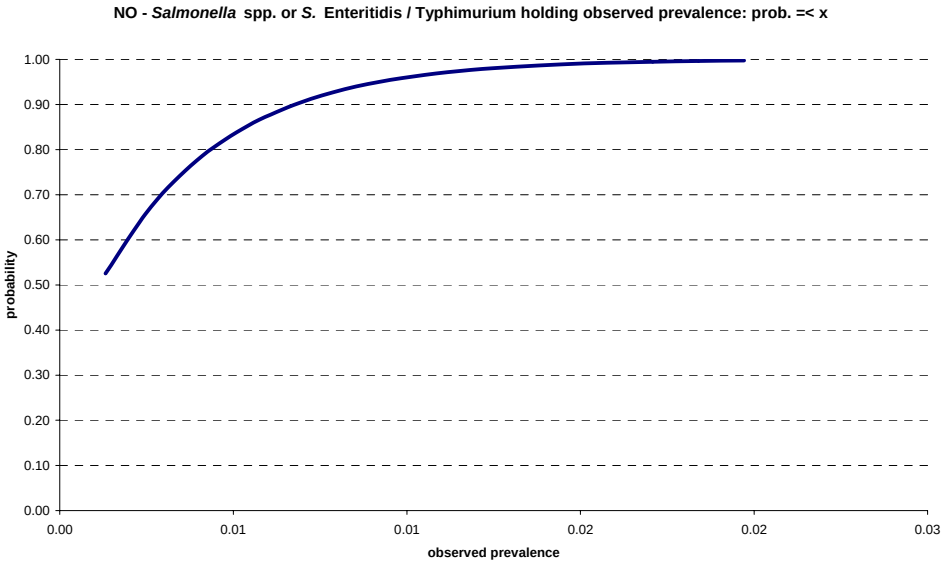
SI - *Salmonella* Enteritidis / Typhimurium holding observed prevalence: prob. =< x



SK - *Salmonella* spp. or *S.* Enteritidis / Typhimurium holding observed prevalence: prob. =< x




UK - Salmonella Enteritidis or Typhimurium holding observed prevalence: prob. =< x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.02
0.04
0.06
0.08
0.10
0.12
observed prevalence


NO - Salmonella spp. or S. Enteritidis / Typhimurium holding observed prevalence: prob. =< x
probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.01
0.01
0.02
0.02
0.03
observed prevalence

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[3] European Commission DG SANCO. Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU: Technical specifications. SANCO/34/2004 Rev3. Working document, 13 July 2004. Presented at the meeting ofthe Standing Committee on the Food Chain and Animal Health on 15 July 2004. (**http://europa.eu.int/comm/food/food/biosafety/salmonella/tech_spec_sanco-34-2004_rev-3_en.pdf**)

[4] European Commission DG SANCO. Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU: Technical specifications. SANCO/34/2004 Rev3. Working document, 13 July 2004. Presented at the meeting ofthe Standing Committee on the Food Chain and Animal Health on 15 July 2004. (**http://europa.eu.int/comm/food/food/biosafety/salmonella/tech_spec_sanco-34-2004_rev-3_en.pdf**)

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European Food Safety Authority, 14 June 2006

Note related to the Preliminary Report on Analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of *Gallus gallus, The EFSA Journal (2006)* 81, 1-71.

This is a preliminary report on the analysis of the Community-wide baseline study to estimate the prevalence of *Salmonella* in laying hen flocks. It is being published pending the full analysis of the entire dataset from the baseline study. The report contains the elements necessary for the establishment of the Community target for reduction of *Salmonella* in laying hens in accordance with Article 4 of Regulation No 2160/2003 of the European Parliament and of the Council on the control of salmonella and other specified food-borne zoonotic agents. Although the final report will not be published until October 2006, key data such as the prevalence levels of salmonella in laying hens is not foreseen to change significantly with the publication of the final report which will contain the full analyses and results from the study. As the European Commission intends to set targets prior to publication of the final report, EFSA is publishing the preliminary analysis in keeping with its policy of openness and transparency.